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Chapter 9

Interleukin-7

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INTRODUCTION

Interleukin-7 (IL-7) is an exceptional cytokine, as it mediates lymphopoiesis in mice in a non-redundant fashion. In contrast, targeted gene deletion of other cytokines, including IL-2, IL-4 or IL-10 (Schorle *et al.*, 1991; Kuhn *et al.*, 1991, 1993), revealed that these cytokines are not essential for development and proper function of B or T lymphocytes. IL-7 is secreted by both immune and non-immune cells and appears not only to be involved in the development of an effective immune system, but also in the generation and maintenance of strong and effective cellular immune responses directed against cancer cells, or infectious diseases. IL-7 appears to serve as the major growth and differentiation factor for both thymic and extrathymic development of $\gamma\delta^+$ T lymphocytes. IL-7 promotes immune effector functions in T lymphocytes, natural killer (NK) cells and monocytes–macrophages, and modulates the quantity and quality of immune responses *in vitro* and *in vivo*. The availability of IL-7 targeted gene-deleted mice, or IL-7 transgenic animals, allowed a more detailed study of the physiology and pathophysiology of the paracrine and systemic effects of IL-7. The use of IL-7 in the treatment of different diseases, including immunodeficiency disorders and malignancy, suggests that IL-7 may facilitate a number of therapeutic endeavors including bone marrow and organ transplantation, cancer immunotherapy and the treatment of infectious diseases.

CLONING AND PURIFICATION

Following the development of techniques for studying bone marrow cultures, it was apparent that B-cell maturation occurred in the presence of bone marrow stromal cells, suggesting the existence of a growth and/or maturation enhancing cytokine (Hunt *et al.*, 1987). Namen and coworkers subsequently demonstrated that conditioned medium from stromal cell cultures stimulated the growth of B-cell precursors. They immortalized a stromal cell line by transfecting it with the plasmid pSV3neo (encoding both the large and small T antigens of SV40) and isolated a clone (I × N/A6) which produced a factor initially called lymphopoietin-1 (LP-1) that stimulated the growth of B-cell precursors.

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Conditioned medium from the growth of this clone was then purified. High-performance liquid chromatography column fractions containing LP-1 bioactivity were isolated. A single unit of LP-1 activity is that causing half maximal ^{3}H-TdR incorporation in a culture of precursor B cells (LP-1 bioassay). At this stage of purification it was clear that several proteins were present in the fraction that could account for the biologic activity. Additional sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis under non-reducing conditions associated bioactivity with a protein of 25×10^3 Da, substantiated by ^{125}I-labeled LP-1 binding experiments. The purified protein exhibits a specific activity of approx. 4×10^6 units/μg of protein and is active at a half-maximal concentration of 10^{-13} M (Namen *et al.*, 1988b).

The same murine stromal cell clone provided a cDNA library which was screened for LP-1 activity following expression in COS-7 cells. A clone (1046) was identified that was associated with high biologic activity (Fig. 1). The sequence contains a 548 base pairs (bp) 5′ non-coding region which may be involved in expression regulation, as its removal results in increased COS cell expression of protein. The sequence includes a 462 bp open reading frame and a 579 bp 3′ non-coding region containing a consensus polyadenylation signal and terminating in 15 adenine residues. Purified protein was subjected to *N*-terminal analysis, which suggested that the nucleotide sequence from clone 1046 codes for the same protein identified in the biologic assay; the protein was designated IL-7. The mature protein has a 25-amino-acid leader sequence followed by 129 amino acids with two *N*-linked glycosylation sites and six cysteine residues which may be involved with intramolecular disulfide bond formation. The importance of disulfide bond formation is suggested by loss of activity following treatment with 2-mercaptoethanol, which breaks disulfide bonds.

The calculated molecular weight of IL-7 is 14.9 kDa. The disparity between calculated molecular weight and that predicted by migration of the native protein may be accounted for by glycosylation (Namen *et al.*, 1988a,b). Two such *N*-linked glycosylation sites in murine IL-7 are located at amino acids 69 and 90 (Namen *et al.*, 1988a). IL-7 mRNA has been detected in murine thymus, spleen, kidney and liver by Northern blot analysis. Interestingly, although message was present in thymus and spleen, no biologic activity could be detected in these tissues.

Goodwin and colleagues characterized human IL-7 by nucleic acid hybridization of cDNA prepared from a hepatocarcinoma cell line (SK-HEP-1, ATCC HTB 52) with the murine IL-7 probe. There is considerable homology between the two IL-7 nucleotide sequences (81% in the coding region) and up to 60% amino acid homology, with all six cysteine residues being conserved (Goodwin *et al.*, 1989). The human IL-7 gene contains six exons over 33 kilobases (kb) (Lupton *et al.*, 1990). The human IL-7 cDNA is composed of 534 nucleotides encoding a protein of 177-amino-acid residues with a signal sequence of 25-amino-acid residues and three potential *N*-linked glycosylation sites (Goodwin *et al.*, 1989). There is a 19-amino-acid insert for human IL-7 (coded for by exon 5 in the human genome) which does not exist in murine IL-7 (Fig. 1) and appears not be essential for biologic IL-7 activity using a proliferation assay of progenitor B cells (Goodwin *et al.*, 1989). Additionally, an apparently alternatively spliced human IL-7 mRNA lacking the entire exon 4 (44-amino-acid residues) was isolated from the SK-HEP-1 line, which results in loss of the capability to stimulate proliferation of murine progenitor B cells. Human recombinant IL-7 is active on murine and human B-cell progenitors. In contrast, murine IL-7 acts only on murine, but not on human cells.

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        -25                      +1       10        20        30        40        50        60        70
hIL-7   MFHVSFRYIFGLPPLILVLLPVASSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDF
hIL-7   MFHVSFRYIFGLPPLILVLLPVASSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANK------------------------
mIL-7   MFHVSFRYIFGIPPLILVLLPVTSSECHIKDKEGKAYESVLMISID-ELDKMTGTDSNCPNNEPNFFRKHVCDDTKEAAFLNRAARKLKQFLKMNISEEF
        exon 1 | exon 2 | exon 3 | exon 4 //

           80        90        100       110       120       130       140       150
IL-7    DLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH
IL-7    -------------------VKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH
mIL-7   NVHLLTVSQGTQTLVNCTSK-------------------EEKNVKEQKK-NDACFLKRLLREIKTCWNKILKGSI--
        // | exon 5 | exon 6 |
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Fig. 1. Amino acid sequences of human and murine IL-7. The hIL-7 gene codes for a 173-amino-acid molecule (top sequence). A differentially spliced IL-7 mRNA has initially been identified (middle sequence) by probing a cDNA library derived from a human hepatocellular carcinoma cell line, with the mIL-7 cDNA by nucleic acid hybridization (Goodwin *et al.*, 1989; Lupton *et al.*, 1990). The alternative IL-7 transcript lacks exon 4 coding for 132 bp, thereby reducing the protein by 44 amino acids. Both the entire IL-7 mRNA and the alternatively spliced IL-7 mRNA have been identified reproducibly in chronic lymphatic B-cell leukemia (Frishman *et al.*, 1993), in follicular dendritic cells (Kröncke *et al.*, 1996a,b) and in renal cell cancer (authors' unpublished observations). The mIL-7 cDNA (bottom sequence) lacks a region that codes for 19 amino acids and would correspond to exon 5 of the human IL-7 gene (Namen *et al.*, 1988a). The lack of exon 5 apparently does not impair biologic IL-7 functions in conventional assay systems. Human and murine IL-7 exhibit up to 81% sequence homology with regard to the nucleotide sequence and up to 60% homology in amino acid residues. The leader peptide is shaded.

receptor structure on T cells according to their state of activation, an observation that may account for differential IL-7-induced signaling events in T cells (Foxwell *et al.*, 1992; Lin *et al.*, 1995). The expression of the 90-kDa IL-7R is stimulated by IL-7, ionomycin and phorbol esters, and inhibited by cyclosporin A and FK506 (Foxwell *et al.*, 1993). Expression of the 90-kDa receptor on freshly isolated human T cells could not be increased with phytohemagglutinin (PHA), concanavalin A or CD3 (Armitage *et al.*, 1992a,b). Interestingly, activation of peripheral blood mononuclear cells (PBMCs) with anti-CD3 results in a fourfold downregulation of IL-7 receptors (high and low affinity) (Foxwell *et al.*, 1992). These findings have recently been substantiated by the observation that IL7Rα–γc chain complexes are detectable in activated, but not in resting, T cells, independent of total cell surface γc chain expression. Thus, stimulation of T cells may lead to assembly of IL7Rα–γc chain complexes, which correlates with JAK3 expression (Page *et al.*, 1997).

However, as IL-2 or IL-4 gene-deleted mice do not exhibit severe defects in T-cell differentiation, such as those observed in either IL-7 or IL-7Rα gene-deleted mice, IL-7 may account for most of the immunologic defects observed in murine models of the X-SCID defect associated with defects of the common γc chain receptor unit (Takeshita *et al.*, 1992; Noguchi *et al.*, 1993; DiSanto *et al.*, 1994; Leonard *et al.*, 1994). The X-SCID defects can also be observed in humans (Lai *et al.*, 1997).

In unstimulated human T cells, the p90 IL-7R is constitutively associated with the Src kinase enzymes $p59^{fyn}$ and $p56^{lck}$ (Page *et al.*, 1995). IL-7 binding the p90 IL-7R leads to both increased $p59^{fyn}$ and $p56^{lck}$ levels in stimulated and unstimulated T cells (Page *et al.*, 1995). Signaling via the p90 IL-7R also leads to increased activity of the Src kinase, suggesting that activation of $p59^{fyn}$ and $p56^{lck}$ is not exclusively responsible for IL-7-driven T-cell proliferation and that other signaling events (e.g. mediated through the γc chain) may be required (Page *et al.*, 1995). However, targeted gene deletion for $p59^{fyn}$ in mice did not show a major impact on lymphopoiesis (Stein *et al.*, 1992; Grabstein *et al.*, 1993; Sudo *et al.*, 1993). In contrast, in $p56^{lck}$ gene-deleted mice, a thymocyte maturation block at the double negative state could be observed (Molina *et al.*, 1992). However, similar effects could not be detected in CD4, CD8, or IL-2 gene-deficient deleted mice. These observations suggest that $p56^{lck}$ is also involved in the signaling pathways (Fung-Leung *et al.*, 1991; Schorle *et al.*, 1991). Thus, the observed effects of $p56^{lck}$ on lymphopoiesis may be attributed to the lack of IL-7-driven $p56^{lck}$-mediated cellular responses. IL-7-mediated phosphatidylinositol-3 (PI-3) kinase activation induced by tyrosine phosphorylation of the PI-3 kinase p85 subunit appears to be to essential to the IL-7 proliferative signal (Sharfe *et al.*, 1995). A different protein tyrosine kinase, termed pim-1, may also be involved in IL-7-mediated signaling, as IL-7-mediated pre-B-cell expansion is decreased in pim-1-deficient mice (Domen *et al.*, 1993).

IL-7 activates members of the Janus (JAK) family of non-receptor tyrosine kinases, JAK1 and JAK3 (Russell *et al.*, 1994; Zeng *et al.*, 1994; Musso *et al.*, 1995), which are both activated by γc chain-sharing cytokines including IL-2, IL-4 and IL-9. These kinases may serve as the signal transduction pathway to the nucleus by phosphorylation and activation of signal transducers and activators of transcription (STATs). IL-7 has been shown to activate STAT1, STAT3 and STAT5 (Zeng *et al.*, 1994; Lin *et al.*, 1995; van der Plas *et al.*, 1996; Perumal *et al.*, 1997) by interacting with an area spanning the tyrosine residue 409 at the *C*-terminal end of the IL-7R (Lin *et al.*, 1995). Thus, at least several alternate signal transduction pathways (e.g. $p56^{lck}$, $p59^{fyn}$, JAKs, STATs) may be

operational in IL-7-responsive cells (e.g. T cells, epithelial cells). It is possible that IL-7 may exert its functions in a cell- or tissue-specific manner dependent on differential activation of the IL-7R signalling transduction pathway(s). For instance, recent data suggest that the IL-7 receptor complex delivers signals of different quality to lymphoid progenitor cells during rearrangement of the antigen receptors (reviewed in Candeias *et al.*, 1997a). First, the IL-7Rα mediates a 'trophic' or 'maintenance' effect regarding cell viability during gene rearrangement. Earlier studies showed that immature thymocytes undergo apoptosis when separated from the thymus. IL-7 is capable of sustaining these cells without inducing significant cell proliferation (Watson *et al.*, 1989). These antiapoptotic effects delivered by the IL-7Rα can also be observed in mature lymphoid cells (Komschlies *et al.*, 1994) and may be attributed to the induction of Bcl-2 members (Hernandez-Caselles *et al*, 1995; Lee *et al.*, 1996; Vella *et al.*, 1997). However, other Bcl-2-related proteins, inducing Bcl-x_L or Bcl-w, or other as yet ill-defined antiapoptotic factors, may also be involved, as bcl-2 knockout $(-/-)$ mice exhibit a different picture concerning T-cell development compared with alterations identified in IL-7R$\alpha^{-/-}$ mice (Veis *et al.*, 1993; Matsuzaki *et al.*, 1997).

Second, the IL-7Rα may also deliver 'mechanistic' signals required for gene rearrangement. IL-7R$\alpha^{-/-}$ mice exhibit impaired γ gene rearrangement (Maki *et al.*, 1996; Candeias *et al.*, 1997b). The same was found to be true for IL-7Rα-mediated signals, required for immunoglobulin (Ig) heavy chain and TCR β-chain rearrangement (Corcoran *et al.*, 1996; Crompton *et al.*, 1997). However, it appears that alternate strategies concerning the IL-7Rα-mediated function may operate in gene rearrangement: the TCR γ-chain rearrangement appears to be dependent on IL-7-mediated signals. Ig heavy chain and TCR-β chain rearrangement requires IL-7, but not absolutely. The TCR δ rearrangement may not be exclusively IL-7 dependent, as IL-7R$\alpha^{-/-}$ mice exhibit δ-chain rearrangements *in vivo* (Corcoran *et al.*, 1996; Candeias *et al.*, 1997a,b; Oosterwegel *et al.*, 1997; Peschon *et al.*, 1997).

Such effects may derive from several factors. First, IL-7 induces RAG-1 (recombinant activation gene) and RAG-2 expression (Muegge *et al.*, 1993). IL-7R$\alpha^{-/-}$ mice exhibit decreased RAG expression in double-negative, but not in double-positive, cells (Crompton *et al.*, 1997). Therefore, decreased recombinase activity may affect recombinatorial events in distinct thymic cells. Second, IL-7Rα-mediated signals may be required to prevent untimely apoptosis in thymocytes. It has been suggested that IL-7Rα-mediated signals may unmask genes associated with proliferation and anti-apoptotic properties (Peschon *et al.*, 1997). This is substantiated by the observation that peripheral T cells in IL-7R$\alpha^{-/-}$ mice undergo apoptosis upon stimulation (Maraskovsky *et al.*, 1996). However, future studies may address in greater detail the antiapoptotic properties and the effects of IL-7Rα-mediated signals on gene rearrangements in immune cells.

IL-7 AND B LYMPHOCYTES

The most compelling evidence that IL-7 represents an important lymphopoietin and possibly one with clinical importance comes from a number of *in vivo* investigations. IL-7 administration to normal mice (5 μg twice daily for 4–7 days) results in a two- to five-fold increase in the number of peripheral and splenic white cells with no significant change in bone marrow cellularity. Analysis of the bone marrow showed an

increase in B-cell precursors (B220$^+$, secretory immunoglobulin (sIg)$^-$) with a concurrent decrease in 8C5 and MAC-1 cells (myelomonocytic marker positive) (Damia *et al.*, 1992).

A general scheme for B-cell maturation is outlined in Table 1. For purposes of clarity, the nomenclature of Hardy and coworkers, defining the early stages of differentiation of murine B cells, has been adapted. These cells can be identified in liver or bone marrow and are divided into distinct classes (A–F) based on cell surface marker expression (Hardy *et al.*, 1991; Li *et al.*, 1993). Adult stem cells develop into 'conventional' B2 cells. Fetal liver stem cells are capable of differentiating into B1 cells, which persist in adult animals, reside primarily into the peritoneal cavity and stain positively for the CD5 antigen. The role of B1 and B2 cells in the context of IL-7 is discussed further below in the section entitled 'IL-7 and antimicrobial immune responses'. The early stages of B-cell development will occur in the bone marrow in response to stromal cell contact and cytokines. Hematopoietic stem cells (HSCs) of the adult bone marrow have been characterized by cell surface marker analysis. HSCs can be derived from murine bone marrow using the CD34 (sialomucin) antibody; other cell surface markers include the antigens CD4, major histocompatibility complex class I, ER-MP12 and AA4.1 (Katz *et al.*, 1985; Berenson *et al.*, 1988; Szilvassy *et al.*, 1989; Wineman *et al.*, 1992; Orlic *et al.*, 1993; Slieker *et al.*, 1993; Szilvassy and Cory, 1993).

Additionally, B-cell differentiation may be defined by DJ or VDJ rearrangement (see Table 1; Hardy *et al.*, 1991; Hardy and Hayakawa, 1991). The antigen receptors of B cells (and those of T cells) are encoded in the germline by individual DNA segments, termed V, D and J, which are joined during lymphocyte differentiation. This process (VDJ recombination) is initiated by the RAG-1 and RAG-2 proteins, which act together at the junctions between the coding segments and the recombination signal sequence to produce two types of DNA ends: a signal end (terminating in a blunt double-stranded break) and a coding end, which terminates in a DNA hairpin. The involvement of double-stranded DNA cleavage has suggested that this process is linked to the cell cycle; several lines of evidence indicate that the initiation of VDJ recombination takes place in the G_0–G_1 phase of the cell cycle (Oettinger *et al.*, 1990; Lewis, 1994). IL-7 appears to sustain expression of the *RAG-1* and *RAG-2* genes (Muegge *et al.*, 1993). The precise mechanism of this process is ill defined. However, more recent data suggest that IL-7 does not alter RAG mRNA levels, but rather affects post-transcriptional regulatory mechanisms. Alternatively, other as yet undefined IL-7-responsive gene products may additionally be involved, as IL-7 appears to be required for induction as well as for maintenance of VDJ recombination. In contrast, IL-7 reduces VDJ recombinatorial events in pre-B cells (Dobbeling, 1996).

To discriminate progenitor cells from cells that are already commited to the B-cell lineage, Hardy and coworkers recently investigated bone marrow stromal cells for expression of the B-cell lineage marker B220 and HSA in combination with the CD4 and AA4.1 markers (Li *et al.*, 1996). The latter marker is expressed on HSCs, B-cell–myeloid progenitors and early B-cell lineage cells (McKearn *et al.*, 1985; Loken *et al.*, 1988; Cumano and Paige, 1992). About 50% of the B220$^+$, CD43$^+$ and HSA$^-$ cells (formerly termed A) stained positive for AA4.1 expression (Li *et al.*, 1996). This cell population was capable of proliferating on a stromal cell layer, indicating that it may indeed represent B-cell lineage precursors. Thus, the earlier designation of fraction-A B cells had to be revised. Two AA4.1 fractions (A_1 and A_2) appear to represent the

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Table 1. IL-7 in B-cell lineage commitment. Cell surface marker expression, V(D)J rearrangement according to Hardy and Hayakawa (1991), Hardy *et al.* (1991), Kitamura *et al.* (1991, 1992), Peschon *et al.* (1994), von Freeden-Jeffrey *et al.* (1995) and Li *et al.* (1996).

Classification	Characteristic cell surface markers	Expression of antigen receptors	IL-7 responsiveness	Anatomic compartment
Pre-pro-B cells: A_0	B220⁻, CD43⁺, AA41⁺, CD4^{low+}	A_0 cells may not yet be lineage committed		Bone marrow
Pre-pro-B cells: A_1	B220⁺, CD43⁺, AA41⁺, CD4^{low+}			Bone marrow
Pre-pro-B cells: A_2	B220⁺, CD43⁺, AA41⁺, CD4^{low-}	Dependent on stromal contact for growth; immunoglobulin genes in germline configuration	Not IL-7 responsive	Bone marrow
Early pro-B cells: B	B220⁺, CD43⁺, AA41⁺, CD4^{low-}, CD19⁺ upregulation of heat-stable antigen(HSA)	DJ rearrangement	Growth in response to IL-7 and stroma	Bone marrow
Late pro-B cells: C	B220⁺, CD43⁺, HSA⁺, CD19⁺	VDJ rearrangement has occurred	IL-7 response in the absence of stroma	Bone marrow, periphery
Pre-B cells: D	B220⁺, CD19⁺, downregulation of CD43 expression		IL-7 alone may stimulate the *in vitro* growth of early pre-B cells, but not late pre-B cells. Development of the small resting B cells requires membrane-bound immunoglobulin heavy chain, λ5 and IL-7	Bone marrow, periphery
Immature B cells: E	B220⁺, CD43⁻, CD19⁺	Light chain rearrangement and detection of IgM on the cell surface (sIgM)		Bone marrow, periphery
Mature B cells: F	B220⁺, CD43⁻, CD19⁺	Encounter of antigen in association with T-cell help may lead to proliferation. Somatic hypermutation of immunoglobulin genes. In response to cytokines, mature B cells undergo immunoglobulin class switching		(Bone marrow), periphery

earliest stages of B-cell lineage development. The B220$^-$, AA4.1$^+$, CD4^{low} fraction has been designated as A_0 cells and appears to represent yet uncommited progenitor cells. However, these 'earliest' stages identified in B-cell development will have to be characterized for activity of B-cell differentiation factors, such as IL-7, kit ligand (Flanagan and Leder, 1990; Williams *et al.*, 1990) and flk2/flt3 ligand (FL) (Matthews *et al.*, 1991; Rosnet *et al.*, 1991). More recent studies have in part addressed this issue. IL-7 does not support *in vitro* growth of cells of the granulocytic–monocytic or erythroid lineage, but does stimulate eosinophil colony formation. This activity can be abolished by anti-IL-5 antibody treatment, suggesting that IL-7 acts by stimulating release of IL-5 or that potentially IL-5 represents an obligate cofactor (Vellenga *et al.*, 1992).

The growth factor combination of IL-11 and mast cell growth factor (MGF) supports bipotential progenitor cells to commit either to the B or to the macrophage lineage (Kee and Paige, 1996). Single-cell cloning assays suggest that IL-7 does not act directly to determine whether cells commit to the B-cell or macrophage lineage. However, bipotential cells respond to IL-7 by an increase in number, and IL-7 added to the IL-11–MGF mixture promotes expression of mRNA transcripts coding for B cell-specific genes (Kee and Paige, 1996). Furthermore, the growth factor combination of IL-11–flt3 ligand–IL-7 appears to maintain the potential of bipotential precursors (Ray *et al.*, 1996). Yet, in a different report, uncommited Lin-SCA-1$^+$ (SCA, stem cell antigen) bone marrow progenitor cells were shown to differentiate into B220$^+$, CD43$^+$, HSA$^+$ B cells (without expressing cytoplasmic μ heavy chain or sIgM) using a combination of flt3 ligand and IL-7 which proved to be superior in driving B-cell differentiation compared with the combination of stem cell factor and IL-7; the latter combination leads to the production of mature granulocytes (Veiby *et al.*, 1996a,b).

Concerning already committed B cells, early pro-B cells require a combination of IL-7 and factors provided by stromal cell layers; late pro-B cells are capable of proliferating in IL-7 without stromal cell support. The same has been found for early pre-B cells, but probably not for late pre-B cells (Hardy *et al.*, 1991; Hardy and Hayakawa, 1991). IL-7-mediated effects in B-cell differentiation may in part be mediated by regulation of the G_1–S transition of the cell cycle (Yasunaga *et al.*, 1995).

Rearrangement of κ light chains and sIgM expression correlates with IL-7Rα downregulation and therefore IL-7 unresponsiveness (Cumano *et al.*, 1990; Park *et al.*, 1990; Era *et al.*, 1991; Henderson *et al.*, 1992). In μ-chain transgenic animals, there is a reduction in the IL-7- and stromal cell-dependent cell population. In animals with a κ-chain transgene, an increase in IL-7-dependent cell populations could be observed. Expression of cytosolic μ chain promotes differentiation to an IL-7-dependent stage. The μ chain-positive cells with a functional light chain gene become IL-7 unresponsive. These results imply that B-cell precursors are driven to the next stage of differentiation by functional immunoglobulin molecules provided by the transgene (Era *et al.*, 1991).

The most precise data concerning the role of IL-7 in B-cell development are provided from IL-7$^{-/-}$ or IL-7R$\alpha^{-/-}$ mice. B lymphopoiesis in bone marrow appeared to be blocked at the transition to pre-B cells (see Table 1). IL-7$^{-/-}$ mice were blocked in the transition between the pro-B (fractions B/C B220$^+$/IgM$^-$/S7$^+$/HSA$^+$) to the pre-B-cell population (fraction D, B220$^+$, IgM$^-$, S7$^-$, HSA$^+$). Thus, differentiation and maturation of B–C fraction B cells to fraction D appears to be IL-7 dependent (Von Freeden-

Jeffrey *et al.*, 1995; Moore *et al.*, 1996). However, IL-7 receptor (IL-7Rα) gene-deleted mice show a block in B-cell development at the transition of pre-pro-B cells (formerly fraction A) to pro-B cells (fraction B) (Peschon *et al.*, 1994). This may be due to the action of other growth factors, potentially the thymic stroma-derived lymphopoietin (TSLP) (Friend *et al.*, 1994; Peschon *et al.*, 1994) or flt3 ligand (Namikawa *et al.*, 1996). Application of IL-7 neutralizing monoclonal antibodies of mice resulted in a similar B-cell maturation blockade to that observed in IL-7Rα knockout animals, but not to the B-cell maturation blockade observed in the IL-7 gene-deleted animals (Grabstein *et al.*, 1993; Peschon *et al.*, 1994). One potential explanation is that other cytokines (e.g. TSLP) may utilize the IL-7 receptor as well. Other cytokines, including TLSP, stem cell factor (SCF)/c-kit or flk2/flt3 ligand (Veiby *et al.*, 1996a,b), may synergize with IL-7 to regulate B-cell development. The SCF–kit ligand, which represents a growth factor for myeloid and erythroid progenitor cells, synergizes with IL-7 in stimulating B-cell precursor cells (McNiece *et al.*, 1991; Billips *et al.*, 1992; Funk *et al.*, 1993). However, some cytokines appear to counteract the IL-7-mediated effects. For instance, IL-1α (Suda *et al.*, 1989), IFN-γ (Garvy and Riley, 1994) and transforming growth factor-β (TGF-β) (Lee *et al.*, 1989) are able to inhibit IL-7-mediated B-cell precursor growth.

Additionally, a number of genes involved in B-cell development may be upregulated by IL-7, including n-*myc*, c-*myc* (Morrow *et al.*, 1992), CD19 (Wolf *et al.*, 1993), the precursor lymphocyte-specific regulatory light chain (PLRLC) (Oltz *et al.*, 1992) and the aminopeptidase BP-1/6C3. Incubation with IL-7 is associated with an increase in 6C3Ag expression by pre-B cells, but not mature B cells. The BP-1/6C3 molecule is expressed by early B-lineage cells and some stromal cells, and represents a type II integral membrane glycoprotein that belongs to the zinc family of metallopeptidases (Sherwood and Weissman, 1990).

In humans, IL-7 does not stimulate proliferation of B-cell lineage cells expressing CD24 (heat-stable antigen). Human pro-B cells but not pre-B cells respond to IL-7 (Ryan *et al.*, 1994; Dittel and LeBien, 1995); this is in contrast to the data for murine cells which suggests that species-specific differences in mode of action exist between humans and mice (Tushinski *et al.*, 1991). This human–rodent dichotomy exists for other cytokines—perhaps most notably IL-4.

In general, human HSC commitment and differentiation has not been as extensively characterized as that of the murine system. However, recent data suggest that certain stages of human B-cell development may not necessarily depend on the presence of IL-7. Using a human bone marrow stromal cell culture system, human HSC CD34 cells underwent commitment, differentiation and expansion into the B-cell lineage as defined by loss of CD34, increased CD19 cell surface expression, and appearance of μ/κ or μ/λ cell surface immunoglobulin receptor expressing immature B cells. This was not significantly influenced either by exogenously added IL-7 or by addition of anti-IL-7 neutralizing antibody (Prieyl and Le Bien, 1996). The implementation of the flt3 ligand in combination with IL-7 or IL-3 using human fetal bone marrow-derived CD34 CD19$^+$ pro-B cells in a stromal cell-independent and serum-deprived culture system revealed that flt3 ligand, like IL-3, synergizes with IL-7 in promoting B-cell growth and differentiation of the majority of cells into CD43$^-$, CD19$^+$, c (cytoplasmic) IgM$^+$, sIgM$^-$ pre-B cells; a minority of pro-B cells matured into sIgM$^+$ B cells (Namikawa *et al.*, 1996). However, the precise role of IL-7 in human B-cell commitment and differentiation has to be analyzed further.

IL-7 AND T LYMPHOCYTES

IL-7 added to murine fetal thymic organ cultures (day 13) causes a preferential expansion of immature cells exhibiting the $CD4^-$, $CD8^-CD3^-$, $CD2^-$, SCA-1^+ phenotype. Cells expressing $\gamma\delta^+$ TCR are increased and the number of $\alpha\beta^+$ TCRs is decreased. Neutralizing anti-IL-7 antibody inhibits growth of fetal thymocytes (Leclercq *et al.*, 1992; Plum *et al.*, 1993). *In vitro* culture of human fetal thymocytes in recombinant IL-7 results in the proliferation of $CD4^+$ and $CD8^+$ thymocytes and partial differentiation of thymocytes with preferential expansion of the $CD4^+$ $CD8^-$ population (Uckun *et al.*, 1991). IL-7 promotes the growth of pre-T cells from fetal liver at day 14 and promotes the expression of TCR-γ, α and β genes (Appasamy, 1992). IL-7 mRNA can be detected in the fetal thymus as early as day 12, peaking at day 15 (Wiles *et al.*, 1992). IL-7 stimulates the generation of $CD3^+$ cells from human bone marrow cultures, with the production of both $CD4^+$ and $CD8^+$ populations (Tushinski *et al.*, 1991). These results suggest that IL-7 may be produced locally in the thymic and bone marrow microenvironments and that it plays a role in the proliferation and potential differentiation of immature T cells (Watanabe *et al.*, 1992). Similar studies have indicated that IL-7 induces the proliferation and maintenance of T-lymphocyte numbers, but not T-cell differentiation. However, with the advent of IL-7, or IL-7Rα gene-deleted mice, several central questions concerning the role of IL-7 in lymphopoiesis could be addressed in more detail.

The macroscopic examination of IL-$7^{-/-}$ mice indicated apparently normal development of both fertile sexes. The lymphatic organs or tissues, including thymus and spleen, were dramatically reduced in size and the peripheral lymph nodes and immune cells within Peyer's patches were not detectable (Von Freeden-Jeffrey *et al.*, 1995). Accordingly, the reduced white blood count in IL-7 gene-deleted mice appeared to be due to an absolute reduction in lymphocytes. However, the normal ratio, as well as the absolute numbers of granulocytes and monocytes, was decreased. Overall, the massive lymphocyte reduction in these animals was due to decreased B- and T-cell numbers (Von Freeden-Jeffrey *et al.*, 1995; Moore *et al.*, 1996), reflecting the inefficient thymic development of IL-7-deficient mice. Only 5% of normal thymocyte numbers and 15% of splenic cell numbers could be detected in IL-7 gene-deleted mice (Von Freeden-Jeffrey *et al.*, 1995; Moore *et al.*, 1996). However, these remaining cells appeared to be similar to those observed in normal mice with regard to function, as defined by testing B cells in response to lipopolysaccharide (LPS), splenic T cells to concanavalin A, or proliferation of thymocytes to a mixture of concanavalin A and IL-2 (Von Freeden-Jeffrey *et al.*, 1995).

Similar T-cell abnormalities to those observed in IL-7 gene-deleted mice have been identified in IL-2Rγ receptor chain knockout mice (Takeshita *et al.*, 1992; Noguchi *et al.*, 1993; DiSanto *et al.*, 1994). As discussed above, the common γc chain is shared by several other cytokines, including IL-2, IL-4, IL-9 and IL-15 (Takeshita *et al.*, 1992; Kondo *et al.*, 1993; Giri *et al.*, 1994). Because IL-2 or IL-4 gene-deleted mice do not exhibit defects in T-cell development, IL-7, but not other cytokines, appears to account for most of the lymphocyte defects observed in murine models of X-SCID associated with abnormalities of the γc chain receptor (Takeshita *et al.*, 1992, 1993; Noguchi *et al.*, 1993; DiSanto *et al.*, 1994).

Thymic T-cell development has been separated into sequential stages based on

expression of distinct cell surface markers. Thymic IL-7 is produced primarily during fetal development (Chantry *et al.*, 1989; Conlon *et al.*, 1989; Okazaki *et al.*, 1989). $CD4^- CD8^-$ fetal and adult immature thymocytes proliferate well in response to IL-7. In contrast, $CD4^+ CD8^+$ thymocytes respond rather poorly. The capability to respond to IL-7 correlates with expression of the IL-7 receptor α chain (IL-7Rα) expressed by $CD4^- CD8^-$, $CD4^+ CD8^-$ and $CD4^- CD8^+$, but not by $CD4^+ CD8^+$ thymocytes (Chantry *et al.*, 1989; Conlon *et al.*, 1989; Okazaki *et al.*, 1989; Everson *et al.*, 1990; Suda and Zlotnik, 1991). Additional studies have indicated that IL-7 mediates effects on TCR rearrangement. T-cell precursors from thymus or fetal liver cultured in IL-7 express rearranged β- or γ-chain transcripts (Appasamy, 1992; Appasamy *et al.*, 1993; Muegge *et al.*, 1993). IL-7, sustaining expression of the *RAG* genes (Muegge *et al.*, 1993; see above) induces rearrangement of Vγ2 and Vγ4, but not Vγ3 or Vγ5, TCR chains in mice (Appasamy *et al.*, 1993). Further evaluation of IL-7 gene-deleted mice showed reduced numbers of total T lymphocytes with preservation of the normal CD4/CD8 ratio and an increased percentage of $\alpha\beta^+$ T cells compared with $\gamma\delta^+$ T cells (Von Freeden-Jeffrey *et al.*, 1995; Moore *et al.*, 1996). These data suggest that proliferation, and not T-cell differentiation, may be affected. However, more recent data indicate that IL-7 may also be involved in T-cell differentiation.

Immature thymocytes have been divided into four distinct phenotypes based on differential expression of the cell surface markers CD25, CD44 and CD117 (c-kit). $CD4^{low}$ cells ($CD44^+$, $CD25^-$, $CD117^+$, $CD3^- CD8^-$) and pro-T cells ($CD44^+$, $CD25^+$, $CD117^+$), representing the early stages of thymic differentiation, are present in $IL7^{-/-}$ mice. In contrast, transition of pro-T cells to pre-T cells ($CD44^- CD25^+$, $CD117^-$) and post-pre-T cells ($CD44^-$, $CD25^-$, $CD117^-$) could not be detected in IL-7 gene-deleted mice (Moore *et al.*, 1995, 1996). Interestingly, lack of IL-7 in such animals resulted in decreased expression of the CD117 (c-kit) marker on $CD4^{low}$ and pro-T cells as well, indicating that IL-7 may induce expression of yet undefined cytokine receptors during thymic T-cell maturation. Based on these data, current models of IL-7-mediated effects may have to be revised, because IL-7 may be critically involved in T-cell differentiation and not only in thymocyte proliferation. However, other thymic factors (e.g. TSLP) may also be critical for thymic differentiation. Future studies may address whether IL-7-deficient animals exhibit a qualitatively different TCR repertoire in peripheral $\alpha\beta^+$ T lymphocytes, particularly in variable TCR chain transcripts which have been shown to be influenced by IL-7 (Appasamy, 1992; Muegge *et al.*, 1993).

More recent studies have scrutinized the role of IL-7 in the development of $\gamma\delta^+$ T lymphocytes. $IL\text{-}7^{-/-}$ mice showed a profound reduction of $CD4^- CD8^- \gamma\delta^+$ T cells to approximately 1% of normal levels (Von Freeden-Jeffrey *et al.*, 1995; Moore *et al.*, 1996). A substantial body of evidence supports the notion that IL-7 preferentially promotes development of $\gamma\delta$ TCR^+ thymocytes over $\alpha\beta^+$ thymocytes, as a result of differential IL-7Rα expression on $\gamma\delta^+$ thymocytes compared with $\alpha\beta^+$ TCR thymocytes. This notion is supported by the fact that $\gamma\delta^+$ T cells are absent in thymus, gut, liver and spleen in $IL\text{-}7R\alpha^{-/-}$ mice (Peschon *et al.*, 1994).

In contrast, $\alpha\beta$ TCR^+ lymphocytes, and NK cells appear to be reduced in number, but to develop normally (He and Malek, 1996; Maki *et al.*, 1996). However, $NK1^+$ T cells can be detected in thymus, liver and spleen of $IL\text{-}7R\alpha^{-/-}$ mice. Recent data suggested that differentiation of these $NK1^+$ cells is dependent of signaling via the γc chain and

expansion on IL-7Rα-mediated signals (Boesteanu *et al.*, 1997). These results provide reasonable evidence that signal transduction mediated by the IL-7 receptor is a prerequisite for $\gamma\delta$ T-cell development in both thymic and extrathymic pathways. Of note, thymocyte development in IL-7R$\alpha^{-/-}$ mice can be reconstituted by the introduction of a transgenic TCR, suggesting that one of several functions of the IL-7Rα may be to initiate TCR gene rearrangement. This notion is further consolidated by the observation that expression of the *RAG-1* and *RAG-2* genes is also significantly reduced in the thymus of IL-7R$\alpha^{-/-}$ mice, but restored in double-positive thymocytes observed in TCR transgenic IL-7R$\alpha^{-/-}$ mice (Crompton *et al.*, 1997). Thus, signaling through the IL-7Rα appears be necessary for *RAG* expression and initiation of VDJ rearrangement, as described for VDJ recombinatorial events in B-cell differentiation (see above). VDJ rearrangement may impact on organ-specific immunity. For instance, pulmonary cells with the canonical fetal-type Vγ6 chain are missing in nude mice owing to a preferred thymic pathway of TCR gene rearrangement, and not to thymic selection. These cells can be restored *in vitro* and *in vivo* by administration of IL-7 (Hayes *et al.*, 1996).

In murine fetal development, T-cell production can be detected at day 15 of gestation. T cells at this stage express the invariant TCR complex composed of Vγ3 and Vδ1 chains. Maturation of thymocytes is accompanied by differential expression of CD24 (heat-stable antigen) expression. First, immature Vγ3 cells exhibit a TCR V$\gamma 3^{low}$ and CD24$^+$ phenotype, and progress to mature V$\gamma 3^{high}$ and CD24$^-$ cells. These $\gamma\delta^+$ T cells may populate the epidermis, or potentially other epithelial sites, and represent the dendritic epidermal T cells (DETCs). Alternatively, $\gamma\delta^+$ T cells may mature extrathymically. Interestingly, IL-7$^{-/-}$ mice characteristically exhibit a block of maturation of V$\gamma 3^{low}$ CD24$^+$ T cells to V$\gamma 3^{high}$ CD24^{low} T cells (Moore *et al.*, 1996). This observation provides another piece of evidence that IL-7 does not serve exclusively as a 'maintenance' factor for thymocytes, but may also be involved in T-cell maturation and differentiation.

In recent years, characterization of T lymphocytes residing primarily in the intestine (intestinal intraepithelial lymphocytes; iIELs) has revealed a distinct phenotype as well as different functional activity for such immune cells compared with 'conventional' $\alpha\beta$ T cells in the periphery (Van Kerckhove *et al.*, 1992; Boismenu and Havran, 1994; Guy-Grand *et al.*, 1994; Havran and Boismenu, 1994; Rocha *et al.*, 1994). Of note, thymic and intestinal epithelial cells share the same embryologic origin as they are both derived from entoderm and may both be capable of secreting IL-7 *in situ* (Namen *et al.*, 1988a; Heufler *et al.*, 1993; Matsue *et al.*, 1993a,b; Ariizumi *et al.*, 1995; Watanabe *et al.*, 1995; Maeurer *et al.*, 1997). Thus, given the fact that IL-7$^{-/-}$ mice (Moore *et al.*, 1995, 1996; Von Freeden-Jeffrey *et al.*, 1995), γc chain knockout mice (Takeshita *et al.*, 1992; Kondo *et al.*, 1993; Noguchi *et al.*, 1993; DiSanto *et al.*, 1994) as well as JAK3-deficient mice (Nosaka *et al.*, 1995; Park *et al.*, 1995) lack $\gamma\delta^+$ T cells, IL-7 appears to represent the major growth/differentiation factor required for thymic and extrathymic development of $\gamma\delta$ T cells. Of note, $\alpha\beta^+$ TCR iIELs are detectable in IL-7$^{-/-}$ mice, but not in γc or in JAK3-deficient mice, suggesting that other cytokines may be critical for generation of $\alpha\beta$ TCR$^+$ iIELs, but not necessarily for $\gamma\delta^+$ TCR iIELs (Takeshita *et al.*, 1992; Noguchi *et al.*, 1993; Di Santo *et al.*, 1994; Moore *et al.*, 1995, 1996; Nosaka *et al.*, 1995; Park *et al.*, 1995; Von Freeden-Jeffrey *et al.*, 1995).

There is strong experimental evidence that TCR$^+$ iIELs may develop *in situ*. Such immune cells are present in both congenitally athymic nude mice and in athymic

radiation chimeras (for review see Poussier and Julius, 1994; Klein, 1996). Much more controversy surrounds the origin of the various subsets of iIELs which show a limited TCR repertoire (Van Kerckhove *et al.*, 1992; Guy-Grand *et al.*, 1994; Poussier and Julius, 1994). IL-7 gene-deleted mice may help to define the impact of IL-7 in the generation and TCR composition of $\alpha\beta^+$ TCR lymphocytes at different anatomic sites, preferentially in the intestine. Recently, clusters of lymphocytes located in crypt lamina propria (designated cryptopatches) have been characterized within the murine small and large intestinal mucosa (Kanamori *et al.*, 1996). Such lymphoid cells are characterized by cell surface expression of CD117$^+$ (c-kit), IL-7Rα^+ and Thy1$^+$, and by the absence of markers for CD3, $\alpha\beta$ TCR, $\gamma\delta$ TCR, sIgM and B220. It has been proposed that the immune cell population first detected at days 14–17 after birth may represent the lymphohematopoietic progenitors for T and B cells in the intestine. The prominent role of IL-7 in lymphopoietic development is further underscored by the observation that such cryptopatch-associated lymphoid cells are virtually absent in IL-7Rα-deficient mice (Kanamori *et al.*, 1996).

IL-7 IN ANTI-MICROBIAL IMMUNE RESPONSES

The role of cytokines in regulation of the host immune response to intracellular and extracellular pathogens has become increasingly understood. Regarding the infection of mice or humans with obligate intracellular pathogens, the T helper cell-1 (T_{H1})-type response, as defined by secretion of IFN-γ, IL-2 and IL-12, appears to represent the principal mediator directed against intracellular infection. Recently, a number of studies have indicated a central role for IL-7 in infections with intracellular bacteria or parasites. Interestingly, IL-7 has a somewhat 'Janus-faced' role, depending on the infection model studied or on the time of IL-7 application in the course of the disease.

Examples of apparently beneficial effects are to be found in murine models of infections with *Mycobacterium* species, or with the parasite *Toxoplasma gondii*. Female A/J mice treated with IL-7, commencing at the time of infection (2 μg daily for 2 weeks) with *T. gondii*, survived. In contrast, mice treated after infection (or not treated) died. *In vivo* depletion experiments have revealed that asialo GM1$^+$ NK cells as well CD8$^+$ T cells are required for protection against the intracellular parasite. Additionally, the IL-7-mediated effects appear to be predominantly mediated by IFN-γ secretion, as *in vivo* depletion of IFN-γ abolished the IL-7 protective effects (Kasper *et al.*, 1995).

In a different infection model, a combination of IL-7 with IL-1β augments anti-*Listeria monocytogenes*-directed immune responses in mice. The cellular immune response is predominantly mediated by peritoneal $\gamma\delta$ T lymphocytes which specifically react to heat-killed *Listeria* preparations in the presence of macrophages as accessory cells in a non-H2-restricted manner. Additionally, the IL-7 responsiveness of $\gamma\delta$ T cells was enhanced in the presence of accessory cells. This effect could be replaced by exogenous IL-1 (Skeen and Ziegler, 1993).

Similarly, IL-7 appears to be involved in the successful immune response directed against infections with mycobacteria. The infection with *Mycobacterium leprae* represents a particular spectrum of the disease, in which the clinical manifestations correlate with the quantity and quality of the cellular immune response. Increased IL-7 mRNA and IL-7 receptor mRNA expression correlates with the tuberculoid form of the disease, in which the infection is limited. In contrast, the lepromatous form, which shows

progressing disease, does not show significant IL-7 mRNA expression (Sieling *et al.*, 1995). Additionally, IL-7 inhibits the intracellular growth of *M. avium complex* (MAC) in human macrophages *in vitro* (Tantawichien *et al.*, 1996). MAC represents a common opportunistic pathogen common in patients with human immunodeficiency virus (HIV) infection. Mononuclear phagocytes represent a major reservoir for MAC in the susceptible host. Such infections are often resistant to standard treatment protocols. Therefore, additional treatment modalities may be required to control MAC infections. For instance, previous studies have shown that treatment of human macrophages with tumor necrosis factor-γ (TNF-α) or granulocyte–macrophage colony stimulating factor (GM-CSF) leads to mycobacteriostatic or mycobactericidal activity (Denis, 1991). Additionally, MAC infection of human macrophages results in the generation of TGF-β, which inhibits the capacity of infected cells to control bacterial growth (Bermudez, 1993). Treatment of human macrophages with IL-7 results in a dose-dependent reduction in the number of intracellular bacteria. When IL-7 was added to cultured macrophages before infection, the anti-MAC activity was diminished compared with that obtained when IL-7 was added to MAC-infected cells (Tantawichien *et al.*, 1996). The authors have obtained similar results with virulent *M. tuberculosis* bacilli.

Treatment of Balb/c mice preinfected with *M. tuberculosis* resulted in up to 100% increased survival compared with that in non-treated mice, or in mice treated with IL-2 or IL-4. These IL-7-mediated effects can be transferred passively, using spleen cells derived from IL-7 treated and *M. tuberculosis*-infected animals, to mice that have been preinfected with mycobacteria. In contrast, transfer of cells from mice treated with IL-7 alone did not result in an increased survival rate compared with control animals, suggesting that priming with *M. tuberculosis* is required to elicit antimicrobial immune responses facilitated by IL-7 treatment (the authors' unpublished observations). In other studies using IL-7 as a treatment, the IL-7-mediated effects could be abolished by antihuman TNF-α antibody. In contrast, IL-7 did not decrease the TGF-β secretion by macrophages upon infection, an observation that was found to be true for IL-7-mediated downregulation of IL-2 or LPS-induced TGF-β mRNA expression in murine macrophages (Dubinett *et al.*, 1993). Therefore, IL-7 may exert some of its effects by inducing or potentiating proinflammatory cytokines, such as IL-1α, IL-1β, IL-6 and TNF-α (Alderson *et al.*, 1991). Additionally, some of the antibactericidal effects of macrophages are mediated by generation of nitric oxide and superoxide radicals, both of which are induced by IL-7 (Alderson *et al.*, 1991; Gessner *et al.*, 1993).

However, IL-7 does not always appear clinically to benefit animals with intracellular infections. For instance, earlier results indicated that IL-7 mediates antimicrobial activity against the intracellular parasite *Leishmania major* in murine macrophages *in vitro* (Gessner *et al.*, 1993). In contrast, treatment of susceptible Balb/c mice with IL-7 at the onset of infection leads to enhanced lesion development and accelerates death of treated animals, correlating with an up to 40-fold increased parasite burden in the spleen and lymph nodes. Analysis of cellular immune responses of such animals has revealed that lymphocytes obtained from IL-7-treated mice produced comparable amounts of the T_{H2} cytokines IL-4 and IL-10, but less IFN-γ in response to antigen (Gessner *et al.*, 1995). This observation suggests that a number of other factors may be involved in the complex interactions of cytokines; for instance, IL-7 upregulates the anti-CD3, or anti-CD3/anti-CD28-induced IFN-γ and IL-4 mRNA expression in (human) T lymphocytes (Borger *et al.*, 1996).

One of the major alterations in the cellular composition of IL-7-treated animals appears to be a rise in total cell numbers in the B-cell compartment. To elucidate the nature of the potentially deleterious B-cell responses, mice with the X-SCID were evaluated. Such animals typically lack B1 cells and exhibit reduced numbers and functions of B2 cells. B1 cells (formerly referred to as Ly-1 B or $CD5^+$ B cells) represent a small subpopulation with a distinct phenotype, and developmental and functional properties. B1 cells express a unique array of cell surface molecules, in addition to expression of the CD5 marker; they are preferentially generated from fetal or neonatal sources of progenitors, and the antibodies derived from B1 cells are predominantly of the IgM class, show minimal hypermutation and a high frequency of low-affinity, poly- or self-reactive specificities (for review see Stall and Wells, 1996). The absence of these cells leads to reduced susceptibility against infections with intracellular parasites (e.g. *Leishmania* species). Of note, following application of a single IL-7 dose concomitant with *Leishmania* infection, the clinical course resembled that in susceptible Balb/mice with an up to 100-fold enhanced parasite load in treated animals. Again, examination of $CD4^+$ *Leishmania*-specific T lymphocytes revealed that IFN-γ secretion is reduced in IL-7-treated Balb/c X-SCID mice compared with that in control animals, and that the population of B2 ($B220^+$, $sIgM^+$, MHC class II^+) cells appeared to be significantly enhanced. However, the nature of the disease-aggravating effects of IL-7 remain to be elucidated. One potential mechanism may be antigen presentation by B cells, an event that may lead to preferential activation and expansion of the T_{H2}-type lymphocytes.

A similar dichotomy of IL-7 has emerged in infection with HIV. Previous studies suggested that exogenous recombinant human IL-7 (rhIL-7) is capable of augmenting the generation of antivirus-directed cytotoxic T-lymphocyte (CTL) responses (Carini *et al.*, 1994). Examination of HIV-infected individuals testing negative for anti-HIV-1-specific CTL reactivity indicated that $CD8^+$ and $CD4^+$ T cells lack IL-7 receptor cell surface expression, which may be attributed to production of insufficient numbers of IL-7R upon retroviral infection or, alternatively, to increased shedding of the IL-7 receptor (Carini and Essex, 1994; Carini *et al.*, 1994). Because HIV infection is accompanied with reduced numbers in the $CD4^+$ cell compartment and associated with loss of cytotoxic $CD8^+$ T-cell activity, several cytokines capable of modulating the immune system have been contemplated for implementation in treatment of HIV-positive individuals. IL-7 is such a candidate (as well as IL-2, IL-12 and IL-15) because it not only enhances anti-HIV-directed $CD8^+$ T-cell responses, but also augments both $CD4^+$ T helper cell-dependent humoral immune responses and $CD8^+$ cytotoxic T-cell reactivity in mice immunized with the HIV envelope protein (Bui *et al.*, 1994).

However, caution must be exercised before implementing these cytokines, including IL-7, into clinical protocols, as exogenous IL-7 induces virus replication and increases proviral DNA levels in PBMC cultures, and increases the levels of doubly spliced HIV-1 TAT RNA (Smithgall *et al.*, 1996). These effects are not inhibited by neutralizing IL-1β, IL-2, IL-6 or TNF-α activity. However, $CD8^+$ T cells inhibit the increase in viral replication induced by IL-7 stimulation, although they do not prevent virus replication following CD3 ligation in the presence of IL-7, an event that can also be mimicked by adding IL-7 to anti-CD3 antibody-stimulated HIV^+ PBMC cultures, resulting in enhanced HIV production (Moran *et al.*, 1993). However, the results obtained from such studies have addressed the role of exogenous IL-7 in HIV replication *in vitro*. They have not addressed the role of endogenous IL-7 on viral load or viremia.

The concentration of IL-7 in plasma from HIV-seronegative individuals as measured by enzyme-linked immunosorbent assay ranges from 1.6 to 9.8 pg/ml (R&D Systems, 1994). These concentrations are 1000-fold lower than the amount of IL-7 implemented for *in vitro* assays. However, there are clinical conditions in which raised IL-7 levels have been determined. For instance, plasma and synovial fluid levels of IL-7 are increased significantly in patients with systemic juvenile rheumatoid arthritis, but not in those with polyarticular or pauciarticular juvenile rheumatoid arthritis, or in patients with other rheumatic diseases (De Benedetti *et al.*, 1995), in patients with untreated Hodgkin's lymphoma (Trumper *et al.*, 1994) and in some with colorectal or renal cell cancer (authors' unpublished observation). However, the precise source and the functional consequence of such increased IL-7 serum levels remain to be determined.

IL-7 mRNA has been observed in a number of different infections, and exogenously added IL-7 (provided either by the recombinant protein or by retroviral infections) has been shown to augment specific cellular immune responses. For instance, IL-7 mRNA has been detected in patients with *Helicobacter pylori*-positive gastritis, but not in *H. pylori*-negative controls (Yamaoka *et al.*, 1995). Others have shown that IL-7 helps the induction of antiviral specific T-cell responses using synthetic peptides and IL-7 as an adjuvant (Kos and Mullbacher, 1992, 1993), or that IL-7 overcomes anergy in parasite-specific cellular immune responses (Sartono *et al.*, 1995), and facilitates expansion of tetanus toxoid (Kim *et al.*, 1994), or dengue virus-specific cytotoxic $CD4^+$ T-cell clones (Berrios *et al.*, 1996).

IL-7 IN 'TISSUE-SPECIFIC' IMMUNITY

Detection of IL-7 mRNA in various tissues has rekindled interest in the role of IL-7 in promoting local immune responses (see Table 2). IL-7 has been reported to be produced by human and murine keratinocytes (Heufler *et al.*, 1993; Matsue *et al.*, 1993a,b; Ariizumi *et al.*, 1995) and serves a major growth factor for dendritic epidermal T cells (DETCs) which express the $\gamma\delta$ TCR (Matsue *et al.*, 1993a,b). The mouse epidermis harbors a T-cell population characterized by expression of CD3, asialo-GM1, CD2, Thy-1, Ly48 and E-cadherin, but not CD4 or CD8 phenotypic markers (Steiner *et al.*, 1988). Such DETCs express the $\gamma\delta$ TCR composed of the $V\gamma3$ and $V\delta1$ chains without junctional diversity (Steiner *et al.*, 1988; Matsue *et al.*, 1993a,b; Moore *et al.*, 1996). These $\gamma\delta$ T-cell effector cells may monitor stressed keratinocytes, or recognize class Ib antigens (e.g. TIa or Qa) (for review see Hayday, 1995). Keratinocytes express constitutively IL-7 mRNA for IL-7 and secrete *in vitro* biologically meaningful amounts of IL-7 protein. IL-7 appears not only to serve as the principal growth factor for DETCs (Fig. 2) but also prevents apoptosis in DETCs exposed to ultraviolet B radiation or corticosteroid treatment (Takashima *et al.*, 1995). This fits well into earlier observations that IL-7 is superior, when compared with IL-2, in maintaining viability and responsiveness in antigen-specific T-cell lines. Additionally, IFN-γ secreted by $\gamma\delta$ T cells appears to inhibit growth of murine keratinocytes (Takashima and Bergstresser, 1996). IL-7 has been shown to augment expression of leukocyte functional antigen-1 (LFA-1) and very late activation antigen-4 in human phorbol myristate acetate (PMA) and calcium-stimulated peripheral blood lymphocytes (PBLs), enhancing the capacity of these cells to adhere to parenchymal cell monolayers (Fratazzi and Carini, 1996). Additionally, IL-7 appears to induce cell surface expression of the costimulatory

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Table 2. IL-7 mRNA or protein detection in cell lines and tissues.

Tissue or cell type	Detection of mRNA	Detection of protein	Reference
Bone marrow stromal cells	+ (h, m)	+ (h, m)	Namen *et al.* (1988a,b) Sudo *et al.* (1989) Witte *et al.* (1993)
Spleen	+ (h, m)	n.d.	Namen *et al.* (1988a,b) Goodwin *et al.* (1989)
Kidney	+ (h, m)	n.d.	Namen *et al.* (1988a,b) Authors' unpublished results
Kidney allograft	+ (h)	n.d.	Strehlau *et al.* (1997)
Renal cell cancer (RCC) tissue sections or RCC cell lines	+ (h)*	+ (h)	Authors' unpublished results
Fetal and adult thymus	+ (h, m)	+ (h, m)	Namen *et al.* (1988a,b) Goodwin *et al.* (1989) Montgomery and Dallman (1991) Sakata *et al.* (1990) Wiles *et al.* (1992) Authors' unpublished results
Thymic stromal cells	+ (m)	+	Sakata *et al.* (1990) Gutierrez and Palacios (1991)
Hassall's corpuscles	+ (h)	n.d.	He *et al.* (1995)
Keratinocytes	+ (h, m)	+ (h, m)	Heufler *et al.* (1993) Matsue *et al.* (1993a,b)
Intestinal epithelial cells, epithelial goblet cells	+ (h)	+ (h)	Watanabe *et al.* (1995)
Colorectal cancer cells	+ (h)*	+ (h)	Maeurer *et al.* (1997) Authors' unpublished results
Uterus	+ (m)	n.d.	Appasamy (1997)
Brain	+ (h)	n.d.	Appasamy (1997)
Adult liver	+ (r)	n.d.	Appasamy (1997)
Hepatocarcinoma	+ (h)*	n.d.	Goodwin *et al.* (1989)
EBV^+ B-cell lines	+ (h)	+ (h)	Benjamin *et al.* (1994)
Burkitt's lymphoma cells	+ (h)	+ (h)	Benjamin *et al.* (1994)
Chronic B-lymphocytic leukemia cells	+ (h)*	+ (h)	Frishman *et al.* (1993) Long *et al.* (1995)
Bladder cancer	+ (h)	n.d.	Kaashoek *et al.* (1991)
Inflammatory malignant fibrous histiocytoma	+ (h)	n.d.	Melhem *et al.* (1993)
Follicular dendritic cells	+ (h)*	+ (h)	Kröncke *et al.* (1996a,b)
Fibroblasts	+ (m)		Aiba *et al.* (1994)
Oral mucosa	+ (h)	+ (h)	Kröncke *et al.* (1996a,b)
Vascular endothelial cells	+ (h)	+ (h)	Kröncke *et al.* (1996a,b)
Hodgkin cell line, nodular sclerosing type	+ (h)	n.d.	Bargou *et al.* (1993)
Sézary lymphoma cells	+ (h)†	n.d.	Foss *et al.* (1994, 1995) Asadullah *et al.* (1996)†
Lesions from tuberculoid lepra	+ (h)	+ (h)	Sieling *et al.* (1995)

n.d., Not determined; h, human; m, mouse; r, rat.
*Cloning and sequence analysis of mRNA exhibits alternatively spliced forms(s) of IL-7; †IL-7 mRNA did not appear to be overexpressed as determined by semiquantitative analysis of skin biopsies from patients with mycosis fungoides or with pleiomorphic T-cell lymphoma compared with biopsies obtained from normal skin, psoriatic lesions or atopic dermatitis.

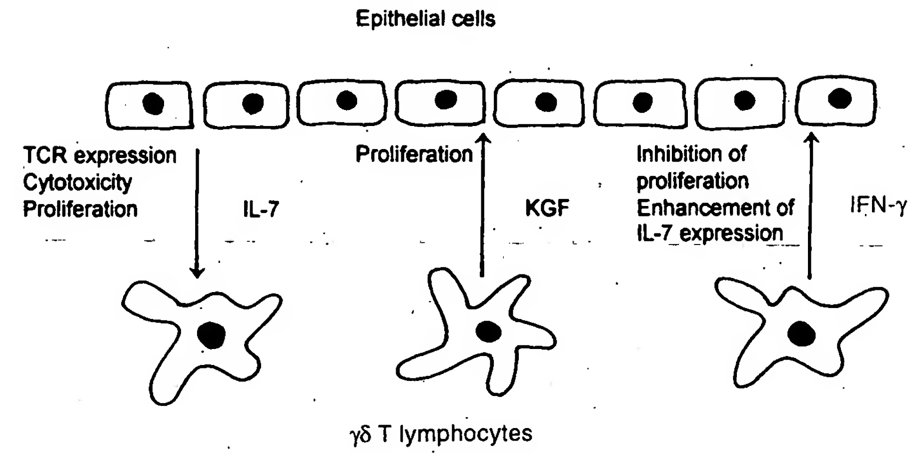

Fig. 2. Tissue-specific immunity: a central role for IL-7 in the interactive milieu of cytokines elaborated by epithelial cells and $\gamma\delta^+$ T lymphocytes. IL-7 secreted by epithelial cells (e.g. keratinocytes) *in situ* is able to maintain viability of $\gamma\delta$ T cells and is critically involved in thymic and extrathymic development of $\gamma\delta$ T cells. IFN-γ produced by $\gamma\delta^+$ T cells alters the IL-7 mRNA transcript pattern in murine keratinocytes (Ariizumi *et al.*, 1995). IFN-γ is able to augment IL-7 secretion by epithelial cells (Ariizumi *et al.*, 1995; Sieling *et al.*, 1995) and inhibits proliferation and immune functions of keratinocytes and downregulates the mitotic capacity of Langerhans cells (Sillevis Smith *et al.*, 1992; Matsue *et al.*, 1993b; Xu *et al.*, 1995). In contrast, keratinocyte growth factor (KGF) secreted by $\gamma\delta^+$ T cells induces proliferation of murine epithelial cells (Boismenu and Havran, 1994).

molecule B7/BB1 as well as intercellular adhesion molecule-1 (ICAM-1) (CD54) on pre-B cells (see Table 1). Acquisition of B7 molecule(s) may be biologically relevant if B cells act as antigen-presenting cells (Dennig and O'Reilly, 1994).

IL-7 as a growth factor for $\gamma\delta$ T cells homing to epidermis represents a critical growth factor in the evolution of contact sensitivity to trinitrochlorbenzene, which can be abrogated by administering monoclonal antibodies to $\gamma\delta$ T cells *in vivo*. $\gamma\delta$ T cells invading the respective antigen challenge site typically exhibit a CD8α^+, CD8β^-, IL-4R$^+$ Vγ3$^+$ phenotype, and proliferate in response to IL-7 but not to IL-2 or IL-4. Moreover, *in vivo* application of IL-7 neutralizing antibody inhibits accumulation of Vγ3$^+$ T cells in the skin, as well as in the regional lymph nodes adjacent to the sensitization site (Dieli *et al.*, 1997). In addition to keratinocytes, other cell types (e.g. fibroblasts) within the epidermis may also provide biologically meaningful IL-7 levels *in vivo* (Aiba *et al.*, 1994). Perhaps the best illustration that IL-7 serves as the major growth factor for IELs is provided by studies from Williams and Kupper, who showed that the epidermal density of DETCs is increased substantially in keratin-14 promoter-driven IL-7 transgenic mice, in which ectopic IL-7 is produced exclusively by keratinocytes (cited as a personal communication in Takashima and Bergstresser, 1996).

The role of IL-7 in skin immune reactions is underscored by the observation that IL-7 mRNA appears to be upregulated in mite allergen patch test reactions in patients with atopic dermatitis (Yamada *et al.*, 1996). The site of positive patch reactions also tested positive for eosinophilic infiltrations. Noteworthy in this context, IL-7 is also capable of upregulating the low-affinity receptor for IgE (CD23) in activated PBLs (Fratazzi and Carini, 1996).

IL-7 may not only be involved in creating an interactive environment of keratinocytes

and $\gamma\delta$ T cells; it may also play a role in the germinal center reaction (Kröncke *et al.*, 1996a). IL-7 mRNA and protein have been detected in human follicular dendritic cells (FDCs) obtained from tonsils. However, mature peripheral IgM^+ B cells are non-responsive to IL-7, whereas anti-μ-stimulated tonsilar B cells proliferate in response to IL-7 without secreting immunoglobulins, suggesting that IL-7 may be indeed be able to regulate B-cell responses in the periphery. In addition to skin and tonsils, IL-7 mRNA and protein have been detected in human intestinal cells (Reinecker and Podolsky, 1995; Watanabe *et al.*, 1995), in human colorectal tumor cells (Maeurer *et al.*, 1997), in normal kidney (Goodwin *et al.*, 1989) and in human renal cell cancer cell lines (authors' unpublished observation). These cells produce significant amounts of IL-7 protein *in vitro*. Of interest, raised IL-7 mRNA expression appears to represent one of the most sensitive markers of graft rejection in patients after kidney transplantation (Strehlau *et al.*, 1997).

IL-7 promotes the growth of lamina propria lymphocytes and inhibits CD3-dependent proliferation of these cells (Watanabe *et al.*, 1995). IL-7, in comparison with IL-2, promotes the preferential expansion of (short term, day 14) cultured tumor-infiltrating lymphocytes obtained from patients with colorectal cancer (Maeurer *et al.*, 1997). Long-term *in vitro* culture of human iIELs harvested from patients with colorectal cancer with IL-7 results in preferential outgrowth of $V\delta1^+$ T cells (Maeurer *et al.*, 1995, 1996) which recognize colorectal cancer cells, renal cell cancer and pancreatic cancer cell lines (Maeurer *et al.*, 1996). Such immune effector cells release significant amounts of IFN-γ. Interestingly, human intestinal cells also appear to express the IL-7Rα. Stimulation of such cells with IL-7 leads to rapid tyrosine phosphorylation of proteins (Reinecker and Podolsky, 1995).

The physiologic role of such IL-7-responsive epithelial cell lines remains to be elucidated. Presumably, IL-7 may represent a member of a family of epithelial growth factors, which promote homing, maturation and maintenance of IL-7-responsive immune cells (see Fig. 2). Thus, IL-7 may be an important cytokine involved in creating an interactive environment of epithelial cells and lymphocytes. Murine $\gamma\delta$ T cells secrete keratinocyte growth factor (KGF), which promotes proliferation of epithelial cells (Boismenu and Havran, 1994). Conversely, human epidermal growth factor (EGF) (Reinecker and Podolsky, 1995) increases IL-7Rα mRNA expression in human colorectal cancer cell lines. Human KGF (authors' unpublished observation) stimulates IL-7 mRNA expression and IL-7 protein secretion by human intestinal cells. Therefore, EGF and/or KGF and IL-7 may represent cytokines involved in the homeostasis of epithelial and immune cells *in vivo* (see Fig. 2). The 'nourishing' capacity of intestinal cells is further substantiated by the observation that bone marrow cells develop into phenotypically mature T cells using a co-culture system with the intestinal epithelial cell line MODE-K (Vidal *et al.*, 1993; Maric *et al.*, 1996). Whether IL-7 is one of the principal factors mediating these effects remains to be determined.

More recently, several reports have addressed the role of IL-7 and IL-7Rα mRNA expression in developing tissues. The observation that IL-7 stimulates maturation of embryonic hippocampal progenitor cells in culture suggests that IL-7 may effect proliferation and differentiation of immature cells of non-hematopoietic origin (Mehler *et al.*, 1993). IL-7 and IL-7R mRNA expression can also be observed in the developing brain, and treatment of culture of embryonic brain with exogenous IL-7 leads to increased neuronal survival and greater numbers of cells exhibiting neurite outgrowth.

One of the IL-7-mediated effects may be via phosphorylation of $p59^{fyn}$ (Michaelson *et al.*, 1996).

IL-7 may also be involved in local immune reactions affecting the eye. The neuroectodermis-derived retinal pigment epithelium (RPE) contributes to the blood–retina barrier regulating infiltration of immune cells in retinal diseases. Activation of RPE cells leads to expression of MHC class II antigens and adhesion molecules. Additionally, IL-7 is able to induce monocyte chemotactic protein-1 and IL-8 in such RPE cells (Elner *et al.*, 1996). However, further studies may address whether IL-7 can be detected in retina-associated diseases *in vivo*.

IL-7 AND CANCER

IL-7 may play different roles in cancer-bearing hosts, dependent on the tumor and status of the immune system. First, IL-7 mRNA, IL-7 protein and the IL-7Rα have been demonstrated in some hematologic malignancies, suggesting that IL-7 may serve as a growth factor in an autocrine fashion. Some tumor cells exhibit expression of the IL-7Rα without IL-7 expression, and may be responsive to IL-7 provided by different cell types. Second, IL-7 may be implemented as a treatment for cancer as IL-7 increases immune effector cell functions by T lymphocytes, NK cells and macrophages. IL-7 may be provided by systemic application, or it may be secreted by genetically engineered tumor cells to induce a strong and long-lived immune response. Third, IL-7 may be one of several growth factors to be used for recovery from bone marrow transplantation in the setting of treatment for hematologic malignancy or, alternatively, for bone marrow rescue after high-dose chemotherapy treatment of solid tumors (e.g. breast cancer).

IL-7 and IL-7Rα Expression in Cancer

IL-7 mRNA and protein expression have been identified in solid tumors including colorectal and renal cell cancers (Watanabe *et al.*, 1995; Maeurer *et al.*, 1997; the authors' unpublished observations). Both tumor cell types express the IL-7Rα receptor and the common γc chain. IL-7 mRNA has also been observed in tumor cells of nodular sclerosing and mixed cellularity type of Hodgkin's disease (Bargou *et al.*, 1993; Foss *et al.*, 1995). The prominent immune cell infiltrate observed in most cases of Hodgkin's disease may be attributed to local delivery of IL-7 *in vivo*. IL-7 serum levels may also be increased in patients with Hodgkin's disease (Trumper *et al.*, 1994; Gorschluter *et al.*, 1995). Similarly, Sézary's lymphoma cells express IL-7Rα and proliferate in response to IL-7. However, some of these lymphoma cells obtained from different patients (3/5) also expressed IL-7 mRNA (Foss *et al.*, 1994).

It has been presumed that keratinocyte-secreted IL-7 may serve as a growth factor for cutaneous T-cell lymphomas. This hypothesis is substantiated by examination of IL-7 transgenic mice. In transgenic mice, in which the IL-7 gene is expressed under the control of the mouse MHC class II (Eα) promoter (Mertsching *et al.*, 1995), a lymphoproliferative syndrome characterized by early polyclonal expansion of T lymphocytes followed by development of pro-pre-B and bipotential myeloid/B-cell tumors can be observed in about 25% of C57Bl/6, and in up to 100% of Balb/c mice (Mertsching *et al.*, 1995, 1996). If the IL-7 gene is controlled by the Sra promoter, which is expressed constitutively in many tissues, development of cutaneous ($\gamma\delta^+$ TCR) lymphomas may be observed

(Uehira *et al.*, 1993). A number of leukemia and lymphoma cells isolated from patients have been screened for their growth responses and/or dependence on IL-7: many but not all proliferate when exposed to IL-7 and the cell types include B- and T-cell malignancies (Eder *et al.*, 1990; Touw *et al.*, 1990; Makrynikola *et al.*, 1991; Shand and Betlach, 1991; Skjonsberg *et al.*, 1991; Lu *et al.*, 1992; Yoshioka *et al.*, 1992). Evidence of lymphoid maturation of the tumor cells in response to IL-7 incubation was not observed (Eder *et al.*, 1990).

In a separate study, pre-B cells transformed by a variety of oncogenes were tested for IL-7 production. None produced any IL-7 bioactivity. IL-7 overexpression achieved by removing portions of the 5′ flanking region was not associated with dramatic colony formation in agar, and most clones were not tumorigenic *in vivo* (syngeneic mice) (Young *et al.*, 1991).

It seems, therefore, that production of IL-7 does not represent a final common step in the malignant transformation of lymphoid cells, but that in selected malignancies it may represent a target for therapeutic intervention. For instance, IL-7 may represent an 'antiapoptotic' factor for some hematopoietic malignancies: IL-7 induces in murine T-cell lymphoma cells (CS-21) expression of the Bcl2 protein and suppression the CPP32-like protease (Lee *et al.*, 1996). An additional role for IL-7 in malignant progression has been suggested by the observation that IL-7 upregulates ICAM expression by melanoma cells, a phenotype correlated with metastatic behavior (for review see Möller *et al.*, 1996).

A detailed study addressed IL-7Rα expression in several types of cutaneous and nodal lymphoma. IL-7Rα was not expressed in cutaneous B-cell lymphomas, benign cutaneous lymphoid infiltrates or reactive lymph nodes. In contrast, IL-7Rα was expressed in over 50% of all histologic types of cutaneous T-cell lymphoma (Bagot *et al.*, 1996). IL-7 mRNA and protein are also readily detectable in B-cell chronic lymphocytic leukemia (B-CLL) cells. The coincidence of IL-7 mRNA downregulation and apoptosis in B-CLL cells suggested that IL-7 gene expression may be required for B-CLL viability *in vivo*. Of note, IL-7 downregulation and apoptosis could be prevented by co-culture of B-CLL cells with human umbilical cord endothelial hybrid cells (EA.hy926). Cell–cell contact appears to be a prerequisite, as cell culture supernatant could not reconstitute the effect, indicating that poorly defined integrins expressed on B-CLL cells may affect IL-7 gene expression and apoptosis (Long *et al.*, 1995). Moreover, IL-7 mRNA and protein elaborated by B-CLL cells may account for some of the clinical symptoms: some patients with CLL may experience suppression of immune responses and also autoimmune symptoms (Frishman *et al.*, 1993). Of note, cloning of the IL-7 gene product from B-CLL cells revealed that at least a different, alternatively spliced, IL-7 mRNA is expressed in tumor cells. The alternatively spliced form appears to be identical with an original IL-7 cDNA clone obtained by screening a (hepatocarcinoma) cDNA library for the human IL-7 gene (Goodwin *et al.*, 1989). The alternative transcript lacks the entire exon 4 (132 bp) coding for 44-amino-acid residues, as depicted in Fig. 1 (Goodwin *et al.*, 1989; Frishman *et al.*, 1993). The present authors have also observed that IL-7 mRNA expressing cells derived from renal or colorectal cancer cells contain the 'canonical' IL-7 full-length IL-7 mRNA and additionally differentially spliced IL-7 mRNA (authors' unpublished observations). The biology of these IL-7 mRNA species remains to be determined.

Based on these data, it appears that IL-7 may exert differential effects on tumor cells. IL-7 may exert growth-promoting, but potentially also growth-arresting, activities. For

instance, proliferation of some pre-B acute lymphoblastic leukemia (B-ALL) cells can be specifically inhibited by exogenous IL-7. This effect can be abrogated by blocking of the IL-7 receptor (Pandrau *et al.*, 1994). In contrast, other ALL cells appear to be IL-7 responsive (Greil *et al.*, 1994). Additionally, targeting of IL-7 receptor-positive cells implementing a recombinant fusion toxin (DAB_{389}–IL-7) has been suggested as a treatment for lymphoma (Sweeney *et al.*, 1995). Thus, IL-7-induced effects mediated by the IL-7Rα may also be dependent on the actual cell type, as proliferation of early pre-B cells (see Table 1) can be augmented by IL-7.

IL-7 as a Treatment Option for Cancer-Bearing Hosts

Immunotherapy has evolved to become a reasonable treatment option for some patients with cancer. This approach, at least theoretically, assumes that an antigenic difference between malignant and normal cells exists, can be recognized by the host and can be manipulated. In addition, it must be presumed that the tumor-bearing host is functionally immunodeficient in that the tumor somehow blocks or inactivates the patient's own antitumor response. For instance, alterations in expression and functions of signal transduction molecules associated with the TCR are indeed responsible for inefficient immune responsiveness in T lymphocytes in several human malignancies, including renal cell cancer, colorectal cancer, ovarian cancer and melanoma.

Decreased CD3 expression and inefficient CD3-mediated signaling in tumor infiltrating lymphocytes (TILs) and PBLs has been observed primarily in tumor-bearing mice (Mizoguchi *et al.*, 1992; Salvadori *et al.*, 1994; Levey and Srivastava, 1995) and recently in cancer-bearing patients as well (Finke *et al.*, 1993; Nakagomi *et al.*, 1993; Matsuda *et al.*, 1995; Tartour *et al.*, 1995; Zea *et al.*, 1995; Lai *et al.*, 1996; Rabinowich *et al.*, 1996).

One of several mechanisms of CD3 downregulation and inefficient signaling appears to be due to reduced expression of the ζ chain of the TCR, presumably related to hydrogen peroxide secretion elaborated by tumor-derived macrophages (Kono *et al.*, 1996). This defect can be reversed *in vitro* and *in vivo* using exogenous IL-2 or IL-2 transfected into tumor cells to be implemented as a vaccine (Salvadori *et al.*, 1994; Rabinowich *et al.*, 1996). However, IL-7 is also capable of upregulating the TCR (Ono *et al.*, 1996) and can enhance protein expression of molecules associated with TCR expression and signaling functions (e.g. ZAP-70, ζ-chain, $p56^{lck}$ and $p59^{fyn}$; authors' unpublished data). Additionally, suppressive factors released by tumors may impair antitumor-directed immune responses, such as TGF-β. Macrophage-derived TGF-β mRNA can be downregulated by IL-7 (Dubinett *et al.*, 1993). The same effect of IL-7 has been found to be true for TGF-β downregulation in a murine fibrosarcoma (Dubinett *et al.*, 1995). In contrast, TGF-β is able to reduce stromal IL-7 mRNA expression and protein secretion using a human *in vitro* lymphoid progenitor cell culture system (Tang *et al.*, 1997). Biologic therapy approaches including immunotherapy seek to reverse this apparent state of anergy and to augment antitumor-directed immune responses.

Clinical trials utilizing IL-2 as a cytokine-based immunotherapy have demonstrated that this approach is successful in treating some patients. The challenge for both clinicians and researchers is to increase the efficacy and decrease the non-specific effects of the therapy. IL-7 appears to have a number of 'IL-2-like' properties and preclinical testing suggests a potential role for IL-7-based immunotherapy trials. The IL-7-mediated effects may be segregated into effects due to non-specific, MHC non-restricted,

lysis of tumor cell targets (e.g. due to lymphokine-activated killer (LAK) cells), MHC class I- or II-specific recognition of tumor cells by $\alpha\beta^+$ T lymphocytes, and tumor-restricted and presumably classic MHC non-restricted recogniton by $\gamma\delta^+$ T-cell effectors.

Characterization of the LAK phenomenon was reported in 1980 by Yron and associates. The phenomenon describes the *in vitro* lysis of labeled fresh tumor targets by lymphoid cells that have been preincubated in IL-2 or other lymphokines. The effect is not MHC restricted and is relatively non-specific, in that a variety of different fresh tumors are lysed yet most normal cells are spared. IL-7 is able to generate LAK activity from thymocytes and PBMCs.

In comparison to IL-2, IL-7 appears to be a relatively weak LAK inducer. IL-2 stimulates fivefold more LAK precursors than IL-7 (Alderson *et al.*, 1990). Thymocytes from cultures grown in IL-2 are highly cytolytic, whereas those grown in IL-7 exhibit minimal cytolytic activity; however, cultures grown in IL-7 and then switched to IL-2 become cytolytic. The addition of IL-4 does not induce cytolytic activity of the cells grown in IL-7 but rather downregulates IL-2-induced proliferation and cytolytic activity (Widmer *et al.*, 1990). IL-7 can generate human LAK cell activity in the absence of IL-2, and induces or upregulates expression of CD25, CD54 and CD69. LAK cell generation is negatively influenced by TGF-β and IL-4. Anti-IL-4 antibody and anti-IL-4 antisense enhances IL-7-induced LAK cell activity (Stötter and Lotze, 1991; Stötter *et al.*, 1991). IL-7 promotes secretion of TNF but not of IFN-γ (Stötter and Lotze, 1991).

The nature of the LAK cell precursor for IL-7-induced LAK is not totally clear. One study showed that LAK cell activity (comparable to IL-2) could be generated from a population of NK cells (CD56$^+$) whereas no LAK activity was generated in PBMCs (Naume and Espevik, 1991; Naume *et al.*, 1992).

Another study using murine cells compared IL-7-induced LAK with IL-2 LAK. IL-7 LAK peaked at day 6–8. IL-7 was more effective at maintaining cytotoxic activity over longer periods of time than IL-2. IL-7 LAK was induced from secondary lymphoid tissue (spleen and nodes) but not from primary lymphoid tissue (thymus and bone marrow). LAK cell activity was abrogated by anti-CD8 or anti-Thy-1 + C and unaffected by anti-CD4, anti-asialo GM-1 or anti-NK-1.1 + C, suggesting that IL-7 LAK may not necessarily be mediated by NK cells, but rather by T lymphocytes (Lynch and Miller, 1990). In comparison to IL-2 and IL-12, IL-7 stimulates the CD56$^+$ NK cells to secrete significantly lower amounts of soluble TNF receptor compared with IL-2-, or IL-12-mediated stimulation. In comparison to IL-2, IL-7 induced lower levels of GM-CSF, but significantly higher GM-CSF levels when compared with IL-12 (Naume *et al.*, 1993).

If indeed, IL-7-induced LAK cell activity resides within the T-cell population, then it might be possible to create a LAK immunotherapy treatment regimen that lacks some of the deleterious effects of the IL-2 treatment which have been blamed on the NK cell population. Circulating human T cells also proliferate when incubated in IL-7. Both CD4$^+$ and CD8$^+$ subsets respond to a similar degree; however, when T cells are separated on the basis of reactivity with an antibody (anti-CD45) that reacts with a 220-kDa isoform (CD45RA) of the common leukocyte antigen, memory T cells (CD45RO) appear to respond more readily than naive T cells (CD45RA) (Welch *et al.*, 1989). Additionally, a variety of effects of IL-7 on monocytes has been reported. Activation of

monocytes with IL-7 can result in the development of a tumor lytic phenotype using melanoma cells as targets (Alderson *et al.*, 1991). Induction of mRNA for both IL-8 and human macrophage inflammatory protein-1 β gene is induced in monocytes by IL-7 (Ziegler *et al.*, 1991; Standiford *et al.*, 1992). Monocytes incubated in IL-7 are stimulated to secrete large quantities of IL-6 as well as IL-1α, IL-1β and TNF-α. This response is abrogated by provision of IL-4.

T cell-based immunotherapy has the advantage of increased specificity compared with LAK cell therapy. T-cell tumor lysis has been shown to be MHC restricted when antimelanoma or renal cell cancer reactive MHC class I- or class II-restricted $\alpha\beta^+$ T lymphocytes are examined. Various cytokines, including IL-2 and IL-4, are active in promoting the clonal expansion *in vitro* of T cells while maintaining their tumor lytic activity. IL-7 appears to have similar properties.

IL-7 alone generates modest CTL activity which is augmented by IL-2, IL-6 or IL-4 (Bertagnolli and Herrmann, 1990; Hickman *et al.*, 1990). Removal of $CD8^+$ cells results in decreased killing, whereas removal of $CD4^+$ cells enhances the CTL response. IL-7 enhances cell proliferation and duration of growth more than IL-2. Allospecific cytotoxicity was maintained for at least 60 days in these cultured cells. Addition of anti-IL-4, anti-IL-2 or anti-IL-6 decreases the proliferation of CTLs in culture (Bertagnolli and Herrmann, 1990; Jicha *et al.*, 1992).

CTLs harvested from draining nodes of tumor-bearing animals and incubated in IL-7 were fourfold more effective than CTLs grown in medium alone in adoptive transfer experiments (Lynch *et al.*, 1991). CTLs incubated in IL-7 and adoptively transferred to mice bearing 3-day pulmonary metastases (MCA tumor) were effective in mediating tumor regression (Jicha *et al.*, 1991).

IL-7 stimulates proliferation of human TILs derived from renal cell carcinoma but only if the TILs are first incubated in either IL-2 alone or in IL-2 plus IL-7. IL-7 stimulates proliferation of $CD4^+$ or $CD8^+$ TIL lines specific for renal cell carcinoma. IL-7 synergizes with anti-CD3 in the induction of IFN-γ from short-term TIL cultures (Sica *et al.*, 1993). Human T cells harvested from peripheral blood and incubated in IL-7 when restimulated with phorbol ester and ionomycin secrete IL-2, IL-4, IL-6 and IFN-γ. This effect was not seen as readily in cultures initiated with IL-2 or IL-4. Both $CD4^+$ and $CD8^+$ subsets responded by cytokine secretion. Almost all the potential to secrete IL-4 and IL-6 in response to IL-7 preincubation resides within the memory subset as opposed to the naive population (Armitage *et al.*, 1992a). The preceding observations suggested that IL-7, either alone or in conjunction with IL-2, acts to stimulate proliferation and tumor lytic activity in sensitized T cells and therefore may be clinically useful in the immunotherapy of malignancy. The most promising data have come from a study demonstrating that anti-tumor-specific T lymphocytes can be grown and expanded *in vitro* without restimulation for extended periods (up to 22 months) compared with T lymphocytes grown in IL-2 (Lynch and Miller, 1994). IL-7 alone (Maeurer *et al.*, 1997), admixture of IL-7 to IL-2 and INF-γ appears also preferentially to expand and maintain tumor-specific and MHC class II-restricted $CD4^+$ T lymphocytes (Cohen *et al.*, 1993) from tumor-bearing patients.

Of note, some of these tumor-reactive and MHC class II-restricted T lymphocytes secrete IFN-γ preferentially in response to autologous tumor cells (Maeurer *et al.*, 1997). This observation is in concordance with an earlier report demonstrating IL-7-mediated effects in adoptive immunotherapy in human colorectal cancer xenografts in SCID mice.

Exclusively the combination of IL-7 treatment and passive transfer of human autologous T cells resulted in enhanced survival of mice engrafted with the respective tumors; treatment with IL-7 alone showed no effect. The antitumor effects are correlated with IFN-γ secretion by the passively transferred T cells and not by their cytolytic capability (Murphy *et al.*, 1993). The ability of IL-7 to generate antitumor-directed immune reactivity may also be dependent on the tumor type and availability of T cells capable of recognizing tumor-associated peptides presented either in the context of MHC class I or II molecules. For instance, application of IL-7 resulted in up to a 75% reduction in pulmonary metastases of the murine renal cell cancer line Renca (Komschlies *et al.*, 1994). However, the pharmacokinetics of IL-7 administered to humans have not yet been evaluated. Some toxic side-effects have been observed in mice treated with IL-7 systemically (Komschlies *et al.*, 1994).

However, IL-7 may also be used to restore the immune system in primary or secondary immunodeficiencies (e.g. induced by viral infections or inherited abnormalities, such as Di George syndrome) or after bone marrow transplantation (BMT). For instance, the successful outcome of autologous BMT is limited by susceptibility to infection. As the effective restitution of an immune system does not only require the quantitative replacement of immune cells (usually achieved within 3–4 months after transplantation), the quality of the immune system is often impaired. Since IL-7 has not only growth-promoting but also differentiation effects on both B- and T-cell lymphopoiesis, it may represent an attractive cytokine, potentially in combination with flt3 ligand, to reconstitute a competent immune system. Several studies have addressed this issue. For instance IL-7 treatment of Balb/c mice after syngeneic BMT leads to increased thymic cellularity, increased RAG-1 expression, and to promotion of Vβ8(D)J gene rearrangement of TCRs. The increased 'quality' of IL-7-treated mice is reflected in better mitogenic responses of thymic cells and in enhanced cytokine production provoked by influenza virus challenge (Abdul-Hai *et al.*, 1996). Additionally, IL-7 accelerates PBL recovery of mice after cyclophosphamide, 5-fluorouracil treatment (Damia *et al.*, 1992) or radiation (Faltynek *et al.*, 1992). Using a metastatic breast cancer model in mice, IL-7 and BMT could significantly prolong survival, presumably due to enhanced immune cell reconstitution after split-dose chemotherapy using cyclophosphamide, cisplatin and nitrosourea (Talmadge *et al.*, 1993).

A more recent study has shown that IL-7 may be able to mobilize long-term reconstituting peripheral CD34^{+} stem cells (Grzegorzewski *et al.*, 1994). Such cells may be useful for stem cell transplantation, or for therapies using CD34^{+} cells either for gene transduction or for maturation *in vitro* in order to generate potent antigen-presenting cells capable of initiating potent antitumor-directed cellular immune responses. Additionally, development of tumors in older individuals may reflect not only accumulative genetic alternations, but also a decreased capacity of the humoral and cellular immune system to identify and eradicate transformed cells efficiently. Several studies have demonstrated age-related alterations in T and B lymphocytes (Zharhary, 1994). In murine models, the ability of pro-B cells to proliferate in response to stroma cells decreases with age (Stephan *et al.*, 1996). This functional alteration is due to an impaired response of pro-B cells to IL-7, but not to other stroma-associated cytokines, including stem cell factor or insulin-like growth factor (Stephan *et al.*, 1997). The reduced IL-7 responsiveness appears not to be induced by inefficient IL-7R

expression, but by as yet poorly defined intracellular signaling events mediated through the IL-7 receptor complex (Stephan *et al.*, 1997). Thus, IL-7 may not only be implemented for primary or secondary immundeficiency disorders: the functional impairment of the immune system in older individuals may in part be mediated by reduced IL-7-responsive immune cells. Future studies may devise therapeutic strategies to overcome age-related immunodeficiencies which may play a role in decreased immune surveillance.

The effects of locally secreted IL-7 elaborated by genetically engineered tumor cells may be overlapping with some of the effects observed by systemic application. Transfection of cytokine genes into tumor cell lines has been developed as a theoretic strategy to increase the local regional response to the tumor in the hope that a heightened *in situ* response might translate to an enhanced systemic response, not only to the transfected tumor but also to the non-transfected or wild-type tumor. IL-7 transfection experiments have yielded some provocative results. Transfection of IL-7 into the murine tumor line J5581 leads to tumor rejection *in vivo*.

$CD8^+$ cells were required for long-term tumor eradication, but short-term regression was noted in the absence of $CD8^+$ cells. While tumor transfected with IL-2, IL-4, TNF or IFN-γ regressed when placed in nude or SCID mice IL-7-transfected tumor required the presence of $CD4^+$ cells for regression, and no regression was observed in nude mice bearing tumor transfected with IL-7.

In most of the murine studies, tumors were eventually rejected by the animals, while the *in vitro* growth was not affected by IL-7 (Hock *et al.*, 1991, 1993; Aoki *et al.*, 1992; McBride *et al.*, 1992; Miller *et al.*, 1993; Allione *et al.*, 1994; Tepper and Mule, 1994). It appears that $CD8^+$ T cells play a major role in mediating tumor rejection (Hock *et al.*, 1991, 1993; Aoki *et al.*, 1992; McBride *et al.*, 1992; Miller *et al.*, 1993) and that antigen-specific T cells are elicited upon immunization with IL-7-secreting tumor cell lines (Aoki *et al.*, 1992). However, other immune cells may also contribute to antitumor responses, as not just T lymphocytes, but also macrophages, eosinophils and basophils, are present at the site of tumor rejection (Hock *et al.*, 1991; McBride *et al.*, 1992).

A more recent study examined in detail the effects of locally secreted IL-7 and induction of tumor-specific cellular immune responses (Cayeux *et al.*, 1995). In B7-transfected mammary adenocarcinoma cells TS/A, T cells showed predominantly $CD28^+$ and $CD25^-$ marker expression, in IL-7-transduced tumor cells CD28 and $CD25^+$ marker expression, whereas in $B7^+IL7^+$ tumor cells the T-cell infiltrate showed typically $CD28^+CD25^+$ expression. The double-transfected tumor elicited a more strong immunity compared with tumor cells expressing IL-7, or B7 alone, or non-transfected tumor cells admixed with *Corynebacterium parvum* (Cayeux *et al.*, 1995). Human non-small-cell lung cancer cell lines infected with a retroviral construct containing the human IL-7 cDNA show alterations of cell surface expression of molecules (e.g. MHC class I, LFA-3) on co-cultured PBLs favoring antitumor-directed immune responses (Sharma *et al.*, 1996). Thus, IL-7-transfected tumor cells may represent a reasonable vaccine for eliciting a strong antitumor-directed immunity (Möller *et al.*, 1996). IL-7-transfected tumor cells are now actively being scrutinized in the setting of tumor vaccines in Heidelberg, Germany (D. Schadendorf) (Möller *et al.*, 1996) and at the University of California at Los Angeles in the USA (J. Economou).

SUMMARY

IL-7 is an important lymphopoietin and plays a critical role in both B- and T-cell development. It promotes expansion of T lymphocytes exhibiting antigen-specific reactivity. IL-7 may be implemented to promote strong and effective immune responses directed against tumor cells, or against microbial or viral infection. It may also be useful to restore an effective and functional immune system after bone marrow transplantation. Clinical trials of IL-7 will begin at the University of Pittsburgh and other institutions soon (supplied by the National Cancer Institute).

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REFERENCES

Abdul-Hai, A., Or, R., Slavin, S., Friedman, G., Weiss, L., Matsa, D. and Ben Yehuda, A. (1996). Stimulation of immune reconstitution by interleukin-7 after syngeneic bone marrow transplantation in mice. *Exp. Hematol.* **24**, 1416–1422.

Aiba, S., Nakagawa, S., Hara, M., Tomioka, Y., Deguchi, M. and Tagami, H. (1994). Cultured murine dermal cells can function like thymic nurse cells. *J. Invest. Dermatol.* **103**, 162–167.

Alderson, M.R., Sassenfeld, H.M. and Widmer, M.B. (1990). Interleukin 7 enhances cytolytic T lymphocyte generation and induces lymphokine-activated killer cells from human peripheral blood. *J. Exp. Med.* **172**, 577–587.

Alderson, M.R., Tough, T.W., Ziegler, S.F. and Grabstein, K.H. (1991). Interleukin 7 induces cytokine secretion and tumoricidal activity by human peripheral blood monocytes. *J. Exp. Med.* **173**, 923–930.

Allione, A., Consalvo, M., Nanni, P., Lollini, P.L., Cavallo, F., Giovarelli, M., Forni, M., Gulino, A., Colombo, M.P. and Dellabona, P. (1994). Immunizing and curative potential of replicating and nonreplicating murine mammary adenocarcinoma cells engineered with interleukin (IL)-2, IL-4, IL-6, IL-7, IL-10, tumor necrosis factor α, granulocyte–macrophage colony-stimulating factor, and γ-interferon gene or admixed with conventional adjuvants. *Cancer Res.* **54**, 6022–6026.

Aoki, T., Tashiro, K., Miyatake, S., Kinashi, T., Nakano, T., Oda, Y., Kikuchi, H. and Honjo, T. (1992). Expression of murine interleukin 7 in a murine glioma cell line results in reduced tumorigenicity *in vivo*. *Proc. Natl Acad. Sci. U.S.A.* **89**, 3850–3854.

Appasamy, P.M. (1992). IL 7-induced T cell receptor-γ gene expression by pre-T cells in murine fetal liver cultures. *J. Immunol.* **149**, 1649–1656.

Appasamy, P.M. (1997). In *Cytokines in Health and Disease* (in press).

Appasamy, P.M., Kenniston, T.W., Jr., Weng, Y., Holt, E.C., Kost, J. and Chambers, W.H. (1993). Interleukin 7-induced expression of specific T cell receptor γ variable region genes in murine fetal liver cultures. *J. Exp. Med.* **178**, 2201–2206.

Ariizumi, K., Meng, Y., Bergstresser, P.R. and Takashima, A. (1995). IFN-γ-dependent IL-7 gene regulation in keratinocytes. *J. Immunol.* **154**, 6031–6039.

Armitage, R.J., Macduff, B.M., Ziegler, S.F. and Grabstein, K.H. (1992a). Multiple cytokine secretion by IL-7-stimulated human T cells. *Cytokine* **4**, 461–469.

Armitage, R.J., Ziegler, S.F., Friend, D.J., Park, L.S. and Fanslow, W.C. (1992b). Identification of a novel low-affinity receptor for human interleukin-7. *Blood* **79**, 1738–1745.

Asadullah, K., Haeussler, A., Friedrich, M., Siegling, A., Olaizola Horn, S., Trefzer, U., Volk, H.D. and Sterry, W. (1996). IL-7 mRNA is not overexpressed in mycosis fungoides and pleomorphic T-cell lymphoma and is likely to be an autocrine growth factor *in vivo*. *Arch. Dermatol. Res.* **289**, 9–13.

Bagot, M., Charue, D., Boulland, M.L., Gaulard, P., Revuz, J., Schmitt, C. and Wechsler, J. (1996). Interleukin-7 receptor expression in cutaneous T-cell lymphomas. *Br. J. Dermatol.* **135**, 572–575.

Bargou, R.C., Mapara, M.Y., Zugck, C., Daniel, P.T., Pawlita, M., Dohner, H. and Dorken, B. (1993). Characterization of a novel Hodgkin cell line, HD-MyZ, with myelomonocytic features mimicking Hodgkin's disease in severe combined immunodeficient mice. *J. Exp. Med*. **177**, 1257–1268.

Benjamin, D., Sharma, V., Knobloch, T.J., Armitage, R.J., Dayton, M.A. and Goodwin, R.G. (1994). B cell IL-7. Human B cell lines constitutively secrete IL-7 and express IL-7 receptors. *J. Immunol*. **152**, 4749–4757.

Berenson, R.J., Andrews, R.G., Bensinger, W.I., Kalamasz, D., Knitter, G., Buckner, C.D. and Bernstein, I.D. (1988). Antigen CD34$^+$ marrow cells engraft lethally irradiated baboons. *J. Clin. Invest*. **81**, 951–955.

Bermudez, L.E. (1993). Production of transforming growth factor-β by *Mycobacterium avium*-infected human macrophages is associated with unresponsiveness to IFN-γ. *J. Immunol*. **150**, 1838–1845.

Berrios, V., Kurane, I. and Ennis, F.A. (1996). Immunomodulatory effects of IL-7 on dengue virus-specific cytotoxic CD4$^+$ T cell clones. *Immunol. Invest*. **25**, 231–240.

Bertagnolli, M. and Herrmann, S. (1990). IL-7 supports the generation of cytotoxic T lymphocytes from thymocytes. Multiple lymphokines required for proliferation and cytotoxicity. *J. Immunol*. **145**, 1706–1712.

Billips, L.G., Petitte, D., Dorshkind, K., Narayanan, R., Chiu, C.P. and Landreth, K.S. (1992). Differential roles of stromal cells, interleukin-7, and kit-ligand in the regulation of B lymphopoiesis. *Blood* **79**, 1185–1192.

Boesteanu, A., De Silva, A.D., Nakajima, H., Leonard, W.J., Peschon, J.J. and Joyce, S. (1997). Distinct roles for signals relayed through the common cytokine receptor γ chain and interleukin 7 receptor α chain in natural T cell development. *J. Exp. Med*. **186**, 331–336.

Boismenu, R. and Havran, W.L. (1994). Modulation of epithelial cell growth by intraepithelial gamma δ T cells. *Science* **266**, 1253–1255.

Borger, P., Kauffman, H.F., Postma, D.S. and Vellenga, E. (1996). IL-7 differentially modulates the expression of IFN-γ and IL-4 in activated human T lymphocytes by transcriptional and post-transcriptional mechanisms. *J. Immunol*. **156**, 1333–1338.

Bui, T., Dykers, T., Hu, S.L., Faltynek, C.R. and Ho, R.J. (1994). Effect of MTP-PE liposomes and interleukin-7 on induction of antibody and cell-mediated immune responses to a recombinant HIV-envelope protein. *J. Acquir. Immune Defic. Syndr*. **7**, 799–806.

Candeias, S., Muegge, K. and Durum, S.K. (1997a). IL-7 receptor and VDJ recombination: trophic *versus* mechanistic actions. *Immunity* **6**, 501–508.

Candeias, S., Peschon, J.J., Muegge, K. and Durum, S.K. (1997b). Defective T-cell receptor γ gene rearrangement in interleukin 7 receptor knockout mice. *Immunol. Lett*. **57**, 9–14.

Carini, C. and Essex, M. (1994). Interleukin 2-independent interleukin 7 activity enhances cytotoxic immune response of. *AIDS Res. Hum. Retroviruses* **10**, 121–130.

Carini, C., McLane, M.F., Mayer, K.H. and Essex, M. (1994). Dysregulation of interleukin-7 receptor may generate loss of cytotoxic T cell response in human immunodeficiency virus type 1 infection. *Eur. J. Immunol*. **24**, 2927–2934.

Cayeux, S., Beck, C., Aicher, A., Dorken, B. and Blankenstein, T. (1995). Tumor cells cotransfected with interleukin-7 and B7.1 genes induce CD25 and CD28 on tumor-infiltrating T lymphocytes and are strong vaccines. *Eur. J. Immunol*. **25**, 2325–2331.

Chantry, D., Turner, M. and Feldmann, M. (1989). Interleukin 7 (murine pre-B cell growth factor/lymphopoietin 1) stimulates thymocyte growth: regulation by transforming growth factor β. *Eur. J. Immunol*. **19**, 783–786.

Cludts, I., Droogmans, L., Cleuter, Y., Kettmann, R. and Burny, A. (1992). Sequence of bovine interleukin 7. *DNA Seq*. **3**, 55–59.

Cohen, P.A., Kim, H., Fowler, D.H., Gress, R.E., Jakobsen, M.K., Alexander, R.B., Mule, J.J., Carter, C. and Rosenberg, S.A. (1993). Use of interleukin-7, interleukin-2, and interferon-γ to propagate CD4$^+$ T cells in culture with maintained antigen specificity. *J. Immunother*. **14**, 242–252.

Conlon, P.J., Morrissey, P.J., Nordan R.P., Grabstein, K.H., Prockett, K.S., Reed, S.G., Goodwin, R., Cosman, D. and Namen, A.E. (1989). Murine thymocytes proliferate in direct response to interleukin 7. *Blood* **74**, 1368–1373.

Corcoran, A.E., Smart, F.M., Cowling, R.J., Crompton, T., Owen, M.J. and Venkitaraman, A.R. (1996). The interleukin-7 receptor α chain transmits distinct signals for proliferation and differentiation during B lymphopoiesis. *EMBO J*. **15**, 1924–1932.

Corey, S.J. and Shapiro, D.N. (1994). Localization of the human gene for Src-related protein tyrosine kinase LYN to chromosome 8q11–12: a lymphoid signaling cluster? *Leukemia* **8**, 1914–1917.

Costello, R., Imbert, J. and Olive, D. (1993). Interleukin-7, a major T-lymphocyte cytokine. *Eur. Cytokine Netw*. **4**, 253–262.

Crompton, T., Outram, S.V., Buckland, J. and Owen, M.J. (1997). A transgenic T cell receptor restores thymocyte differentiation in interleukin-7 receptor α chain-deficient mice. *Eur. J. Immunol.* **27**, 100–104.

Cumano, A. and Paige, C.J. (1992). Enrichment and characterization of uncommitted B-cell precursors from fetal liver at day 12 of gestation. *EMBO J.* **11**, 593–601.

Cumano, A., Dorshkind, K., Gillis, S. and Paige, C.J. (1990). The influence of S17 stromal cells and interleukin 7 on B cell development. *Eur. J. Immunol.* **20**, 2183–2189.

Damia, G., Komschlies, K.L., Faltynek, C.R., Ruscetti, F.W. and Wiltrout, R.H. (1992). Administration of recombinant human interleukin-7 alters the frequency and number of myeloid progenitor cells in the bone marrow and spleen of mice. *Blood* **79**, 1121–1129.

De Benedetti, F., Massa, M., Pignatti, P., Kelley, M., Faltynek, C.R. and Martini, A. (1995). Elevated circulating interleukin-7 levels in patients with systemic juvenile rheumatoid arthritis. *J. Rheumatol.* **22**, 1581–1585.

Denis, M. (1991). Tumor necrosis factor and granulocyte macrophage-colony stimulating factor stimulate human macrophages to restrict growth of virulent *Mycobacterium avium* and to kill avirulent *M. avium*: killing effector mechanism depends on the generation of reactive nitrogen intermediates. *J. Leukoc. Biol.* **49**, 380–387.

Dennig, D. and O'Reilly, R.J. (1994). IL-7 induces surface expression of B7/BB1 on pre-B cells and an associated increase in their costimulatory effects on T cell proliferation. *Cell Immunol.* **153**, 227–238.

Dieli, F., Asherson, G.L., Sireci, G., Dominici, R., Gervasi, F., Vendetti, S., Colizzi, V. and Salerno, A. (1997). $\gamma\delta$ cells involved in contact sensitivity preferentially rearrange the Vγ3 region and require interleukin-7. *Eur. J. Immunol.* **27**, 206–214.

DiSanto, J.P., Muller, W., Guy Grand, D., Fischer, A. and Rajewsky, K. (1995). Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor γ chain. *Proc. Natl Acad. Sci. U.S.A.* **92**, 377–381.

Dittel, B.N. and LeBien, T.W. (1995). The growth response to IL-7 during normal human B cell ontogeny is restricted to B-lineage cells expressing CD34. *J. Immunol.* **154**, 58–67.

Dobbeling, U. (1996). The influence of IL-7 V(D)J recombination. *Immunology* **89**, 569–572.

Domen, J., van der Lugt, N.M., Acton, D., Laird, P.W., Linders, K. and Berns, A. (1993). Pim-1 levels determine the size of early B lymphoid compartments in bone marrow. *J. Exp. Med.* **178**, 1665–1673.

Dubinett, S.M., Huang, M., Dhanani, S., Wang, J. and Beroiza, T. (1993). Down-regulation of macrophage transforming growth factor-β messenger RNA expression by IL-7. *J. Immunol.* **151**, 6670–6680.

Dubinett, S.M., Huang, M., Dhanani, S., Economou, J.S., Wang, J., Lee, P., Sharma, S., Dougherty, G.J. and McBride, W.H. (1995). Down-regulation of murine fibrosarcoma transforming growth factor-β_1 expression by interleukin 7. *J. Natl Cancer Inst.* **87**, 593–597.

Eder, M., Ottmann, O.G., Hausen Hagge, T.E., Bartram, C.R., Gillis, S., Hoelzer, D. and Gauser, A. (1990). *Leukemia* **4**, 533–540.

Elner, V.M., Elner, S.G., Standiford, T.J., Lukacs, N.W., Strieter, R.M. and Kunkel, S.L. (1996). Interleukin-7 (IL-7) induces retinal pigment epithelial cell MCP-1 and IL-8. *Exp. Eye Res.* **63**, 297–303.

Era, T., Ogawa, M., Nishikawa, S., Okamoto, M., Honjo, T., Akagi, K., Miyazaki, J. and Yamamura, K. (1991). Differentiation of growth signal requirement of B lymphocyte precursor is directed by expression of immunoglobulin. *EMBO J.* **10**, 337–342.

Everson, M.P., Eldridge, J.H. and Koopman, W.J. (1990). Synergism of interleukin 7 with the thymocyte growth factors interleukin 2, interleukin 6, and tumor necrosis factor α in the induction of thymocyte proliferation. *Cell. Immunol.* **127**, 470–482.

Faltynek, C.R., Wang, S., Miller, D., Young, E., Tiberio, L., Kross, K., Kelley, M. and Kloszewski, E. (1992). Administration of human recombinant IL-7 to normal and irradiated mice increases the numbers of lymphocytes and some immature cells of the myeloid lineage. *J. Immunol.* **149**, 1276–1282.

Finke, J.H., Zea, A.H., Stanley, J., Longo, D.L., Mizoguchi, H., Tubbs, R.R., Wiltrout, R.H., O'Shea, J.J., Kudoh, S., Klein, E., Bukowski, R.M. and Ochoa, A. (1993). Loss of T-cell receptor zeta chain and p56lck in T-cells infiltrating human renal cell carcinoma. *Cancer Res.* **53**, 5613–5616.

Flanagan, J.G. and Leder, P. (1990). The kit ligand: a cell surface molecule altered in Steel mutant fibroblasts. *Cell* **63**, 185–194.

Foss, F.M., Koc, Y., Stetler Stevenson, M.A., Nguyen, D.T., O'Brien, M.C., Turner, R. and Sausville, E.A. (1994). Costimulation of cutaneous T-cell lymphoma cells by interleukin-7 and interleukin-2: potential autocrine or paracrine effectors in the Sézary syndrome. *J. Clin. Oncol.* **12**, 326–335.

Foss, H.D., Hummel, M., Gottstein, S., Ziemann, K., Falini, B., Herbst, H. and Stein, H. (1995). Frequent expression of IL-7 gene transcripts in tumor cells of classical Hodgkin's disease. *Am. J. Pathol.* **146**, 33–39.

Foxwell, B.M., Taylor Fishwick, D.A., Simon, J.L., Page, T.H. and Londei, M. (1992). Activation induced changes in expression and structure of the IL-7 receptor on human T cells. *Int. Immunol.* **4**, 277–282.

Foxwell, B.M., Willcocks, J.L., Taylor Fishwick, D.A., Kulig, K., Ryffel, B. and Londei, M. (1993). *Eur. J. Immunol.* **23**, 85–89.

Fratazzi, C. and Carini, C. (1996). Interleukin-7 modulates intracytoplasmatic CD23 production and induces adhesion molecule expression and adhesiveness in activated $CD4^{+}CD23^{+}$ T cell subsets. *Clin. Immunol. Immunopathol.* **81**, 261–270.

Friend, S.L., Hosier, S., Nelson, A., Foxworthe, D., Williams, D.E. and Farr, A. (1994). A thymic stromal cell line supports *in vitro* development of surface IgM^{+} B cells and produces a novel growth factor affecting B and T lineage cells. *Exp. Hematol.* **22**, 321–328.

Frishman, J., Long, B., Knospe, W., Gregory, S. and Plate, J. (1993). Genes for interleukin 7 are transcribed in leukemic cell subsets of individuals with chronic lymphocytic leukemia. *J. Exp. Med.* **177**, 955–964.

Fujihashi, K., Kawabata, S., Hiroi, T., Yamamoto, M., McGhee, J.R., Nishikawa, S. and Kiyono, H. (1996). Interleukin 2 (IL-2) and interleukin 7 (IL-7) reciprocally induce IL-7 and IL-2 receptors on $\gamma\delta$ T-cell receptor-positive intraepithelial lymphocytes. *Proc. Natl Acad. Sci. U.S.A.* **93**, 3613–3618.

Fung Leung, W.P., Schilham, M.W., Rahemtulla, A., Kundig, T.M., Vollenweider, M., Potter, J., van Ewijk, W. and Mak, T.W. (1991). CD8 is needed for development of cytotoxic T cells but not helper T cells. *Cell* **65**, 443–449.

Funk, P.E., Varas, A. and Witte, P.L. (1993). Activity of stem cell factor and IL-7 in combination on normal bone marrow B lineage cells. *J. Immunol.* **150**, 748–752.

Garvy, B.A. and Riley, R.L. (1994). IFN-γ abrogates IL-7-dependent proliferation in pre-B cells, coinciding with onset of apoptosis. *Immunology* **81**, 381–388.

Gessner, A., Vieth, M., Will, A., Schroppel, K. and Rollinghoff, M. (1993). Interleukin-7 enhances antimicrobial activity against *Leishmania major* in murine macrophages. *Infect. Immun.* **61**, 4008–4012.

Gessner, A., Will, A., Vieth, M., Schroppel, K. and Rollinghoff, M. (1995). Stimulation of B-cell lymphopoiesis by interleukin-7 leads to aggravation of murine leishmaniasis. *Immunology* **84**, 416–422.

Giri, J.G., Ahdieh, M., Eisenman, J., Shanebeck, K., Grabstein, K., Kumaki, S., Namen, A., Park, L.S., Cosman, D. and Anderson, D. (1994). Utilization of the β and γ chains of the IL-2 receptor by the novel cytokine IL-15. *EMBO J.* **13**, 2822–2830.

Goodwin, R.G., Lupton, S., Schmierer, A., Hjerrild, K.J., Jerzy, R., Clevenger, W., Gillis, S., Cosman, D. and Namen, A.E. (1989). Human interleukin 7: molecular cloning and growth factor activity on human and murine B-lineage cells. *Proc. Natl Acad. Sci. U.S.A.* **86**, 302–306.

Goodwin, R.G., Friend, D., Ziegler, S.F., Jerzy, R., Falk, B.A., Gimpel, S., Cosman, D., Dower, S.K., March, C.J. and Namen, A.E. (1990). Cloning of the human and murine interleukin-7 receptors: demonstration of a soluble form and homology to a new receptor superfamily. *Cell* **60**, 941–951.

Gorschluter, M., Bohlen, H., Hasenclever, D., Diehl, V. and Tesch, H. (1995). Serum cytokine levels correlate with clinical parameters in Hodgkin's disease. *Ann. Oncol.* **6**, 477–482.

Grabstein, K.H., Waldschmidt, T.J., Finkelman, F.D., Hess, B.W., Alpert, A.R., Boiani, N.E., Namen, A.E. and Morrissey, P.J. (1993). Inhibition of murine B and T lymphopoiesis *in vivo* by an anti-interleukin 7 monoclonal antibody. *J. Exp. Med.* **178**, 257–264.

Greil, J., Gramatzki, M., Burger, R., Marschalek, R., Peltner, M., Trautmann, U., Hansen Hagge, T.E., Bartram, C.R., Fey, G.H. and Stehr, K. (1994). The acute lymphoblastic leukaemia cell line SEM with t(4;11). chromosomal rearrangement is biphenotypic and responsive to interleukin-7. *Br. J. Haematol.* **86**, 275–283.

Grzegorzewski, K., Komschlies, K.L., Mori, M., Kaneda, K., Usui, N., Faltynek, C.R., Keller, J.R., Ruscetti, F.W. and Wiltrout, R.H. (1994). Administration of recombinant human interleukin-7 to mice induces the exportation of myeloid progenitor cells from the bone marrow to peripheral sites. *Blood* **83**, 377–385.

Gutierrez, J.C. and Palacios, R. (1991). Heterogeneity of thymic epithelial cells in promoting T-lymphocyte differentiation *in vivo*. *Proc. Natl Acad. Sci. U.S.A.* **88**, 642–646.

Guy Grand, D., Rocha, B., Mintz, P., Malassis Seris, M., Selz, F., Malissen, B. and Vassalli, P. (1994). Different use of T cell receptor transducing modules in two populations of gut intraepithelial lymphocytes are related to distinct pathways of T cell differentiation. *J. Exp. Med.* **180**, 673–679.

Hardy, R.R. and Hayakawa, K. (1991). A developmental switch in B lymphopoiesis. *Proc. Natl Acad. Sci. U.S.A.* **88**, 11 550–11 554.

Hardy, R.R., Carmack, C.E., Shinton, S.A., Kemp, J.D. and Hayakawa, K. (1991). Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow. *J. Exp. Med.* **173**, 1213–1225.

Havran, W.L. and Boismenu, R. (1994). Activation and function of $\gamma\delta$ T cells. *Curr. Opin. Immunol.* **6**, 442–446.

Hayday, A.C. (1995). $\gamma\delta$ T cell specificity and function. In *T Cell Receptors* (eds J.I. Bell, M.J. Owen and E. Simpson), Oxford University Press, Oxford, pp. 70–91.

Hayes, S.M., Sirr, A., Jacob, S., Sim, G.K. and Augustin, A. (1996). Role of IL-7 in the shaping of the pulmonary $\gamma\delta$ T cell repertoire. *J. Immunol.* **156**, 2723–2729.

He, Y.W., Adkins, B., Furse, R.K. and Malek, T.R. (1995). Expression and function of the γc subunit of the IL-2, IL-4, and IL-7 receptors. Distinct interaction of γc in the IL-4 receptor. *J. Immunol.* **154**, 1596–1605.

He, Y.W. and Malek, T.R. (1996). Interleukin-7 receptor α is essential for the development of $\gamma\delta^+$ T cells, but not natural killer cells. *J. Exp. Med.* **184**, 289–293.

Henderson, A.J., Narayanan, R., Collins, L. and Dorshkind, K. (1992). Status of κ L chain gene rearrangements and c-kit and IL-7 receptor expression in stromal cell-dependent pre-B cells. *J. Immunol.* **149**, 1973–1979.

Hernandez Caselles, T., Martinez Esparza, M., Sancho, D., Rubio, G. and Aparicio, P. (1995). Interleukin-7 rescues human activated T lymphocytes from apoptosis induced by glucocorticoesteroids and regulates bcl-2 and CD25 expression. *Hum. Immunol.* **43**, 181–189.

Heufler, C., Topar, G., Grasseger, A., Stanzl, U., Koch, F., Romani, N., Namen, A.E. and Schuler, G. (1993). Interleukin 7 is produced by murine and human keratinocytes. *J. Exp. Med.* **178**, 1109–1114.

Hickman, C.J., Crim, J.A., Mostowski, H.S. and Siegel, J.P. (1990). Regulation of human cytotoxic T lymphocyte development by IL-7. *J. Immunol.* **145**, 2415–2420.

Hock, H., Dorsch, M., Diamantstein, T. and Blankenstein, T. (1991). Interleukin 7 induces $CD4^+$ T cell-dependent tumor rejection. *J. Exp. Med.* **174**, 1291–1298.

Hock, H., Dorsch, M., Kunzendorf, U., Qin, Z., Diamantstein, T. and Blankenstein, T. (1993). Mechanisms of rejection induced by tumor cell-targeted gene transfer of interleukin 2, interleukin 4, interleukin 7, tumor necrosis factor, or interferon γ. *Proc. Natl Acad. Sci. U.S.A.* **90**, 2774–2778.

Hunt, P., Robertson, D., Weiss, D., Rennick, D., Lee, F. and Witte, O.N. (1987). A single bone marrow-derived stromal cell type supports the *in vitro* growth of early lymphoid and myeloid cells. *Cell* **48**, 997–1007.

Jicha, D.L., Mule, J.J. and Rosenberg, S.A. (1991). Interleukin 7 generates antitumor cytotoxic T lymphocytes against murine sarcomas with efficacy in cellular adoptive immunotherapy. *J. Exp. Med.* **174**, 1511–1515.

Jicha, D.L., Schwarz, S., Mule, J.J. and Rosenberg, S.A. (1992). Interleukin-7 mediates the generation and expansion of murine allosensitized and antitumor CTL. *Cell Immunol.* **141**, 71–83.

Kaashoek, J.G., Mout, R., Falkenburg, J.H., Willemze, R., Fibbe, W.E. and Landegent, J.E. (1991). Cytokine production by the bladder carcinoma cell line 5637: rapid analysis of mRNA expression levels using a cDNA–PCR procedure. *Lymphokine Cytokine Res.* **10**, 231–235.

Kanamori, Y., Ishimaru, K., Nanno, M., Maki, K., Ikuta, K., Nariuchi, H. and Ishikawa, H. (1996). Identification of novel lymphoid tissues in murine intestinal mucosa where clusters of c-kit$^+$ IL-7R$^+$ Thy1$^+$ lympho-hemopoietic progenitors develop. *J. Exp. Med.* **184**, 1449–1459.

Kasper, L.H., Matsuura, T. and Khan, I.A. (1995). IL-7 stimulates protective immunity in mice against the intracellular pathogen, *Toxoplasma gondii*. *J. Immunol.* **155**, 4798–4804.

Katz, F.E., Tindle, R., Sutherland, D.R. and Greaves, M.F. (1985). Identification of a membrane glycoprotein associated with haemopoietic progenitor cells. *Leuk. Res.* **9**, 191–198.

Kee, B.L. and Paige, C.J. (1996). *In vitro* tracking of IL-7 responsiveness and gene expression during commitment of bipotent B-cell/macrophage progenitors. *Curr. Biol.* **6**, 1159–1169.

Kim, J.H., Ratto, S., Sitz, K.V., Mosca, J.D., McLinden, R.J., Tencer, K.L., Vahey, M.T., St Louis, D., Birx, D.L. and Redfield, R.R. (1994). Consequences of stable transduction and antigen-inducible expression of the human interleukin-7 gene on tetanus-toxoid-specific T cells. *Hum. Gene Ther.* **5**, 1457–1466.

Kishimoto, T., Goyert, S., Kikutani, H., Mason, D., Miyasaka, M., Moretta, L., Ohno, T., Okumura, K., Shaw, S., Springer, T.A., Sugamura, K., Sugawara, H., von dem Borne, A.E. and Zola, H. (1997). Update of CD antigens, 1996. *J. Immunol.* **158**, 3035–3036.

Kitamura, D., Roes, J., Kuhn, R. and Rajewsky, K. (1991). A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. *Nature* **350**, 423–426.

Kitamura, D., Kudo, A., Schaal, S., Muller, W., Melchers, F. and Rajewsky, K. (1992). A critical role of λ5 protein in B cell development. *Cell* **69**, 823–831.

Klein, J.R. (1996). Whence the intestinal intraepithelial lymphocyte? *J. Exp. Med.* **184**, 1203–1206.

Komschlies, K.L., Gregorio, T.A., Gruys, M.E., Back, T.C., Faltynek, C.R. and Wiltrout, R.H. (1994). Administration of recombinant human IL-7 to mice alters the composition of B-lineage cells and T cell subsets, enhances T cell function, and induces regression of established metastases. *J. Immunol.* **152**, 5776–5784.

Kondo, M., Takeshita, T., Ishii, N., Nakamura, M., Watanabe, S., Arai, K. and Sugamura, K. (1993). Sharing of the interleukin-2 (IL-2) receptor γ chain between receptors for IL-2 and IL-4. *Science* **262**, 1874–1877.

Kondo, M., Takeshita, T., Higuchi, M., Nakamura, M., Sudo, T., Nishikawa, S. and Sugamura, K. (1994). Functional participation of the IL-2 receptor γ chain in IL-7 receptor complexes. *Science* **263**, 1453–1454.

Kono, K., Salazar Onfray, F., Petersson, M., Hansson, J., Masucci, G., Wasserman, K., Nakazawa, T., Anderson, P. and Kiessling, R. (1996). Hydrogen peroxide secreted by tumor-derived macrophages down-modulates signal-transducing zeta molecules and inhibits tumor-specific T cell- and natural killer cell-mediated cytotoxicity. *Eur. J. Immunol*. **26**, 1308–1313.

Kos, F.J. and Mullbacher, A. (1992). Induction of primary anti-viral cytotoxic T cells by *in vitro* stimulation with short synthetic peptide and interleukin-7. *Eur. J. Immunol*. **22**, 3183–3185.

Kos, F.J. and Mullbacher, A. (1993). IL-2-independent activity of IL-7 in the generation of secondary antigen-specific cytotoxic T cell responses *in vitro*. *J. Immunol*. **150**, 387–393.

Kröncke, R., Loppnow, H., Flad, H.D. and Gerdes, J. (1996a). Human follicular dendritic cells and vascular cells produce interleukin-7: a potential role for interleukin-7 in the germinal center reaction. *Eur. J. Immunol*. **26**, 2541–2544.

Kröncke, R., Steffen, S., Flad, H.-D. and Gerdes, J. (1996b). IL-7 mRNA exists in different alternatively spliced isoforms. *Immunobiology* **196**, 12 (Abstract A18).

Kuhn, R., Rajewsky, K. and Muller, W. (1991). Generation and analysis of interleukin-4 deficient mice. *Science* **254**, 707–710.

Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K. and Muller, W. (1993). Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* **75**, 263–274.

Lai, P., Rabinowich, H., Crowley-Nowick, P.A., Bell, M.C., Mantovani, G. and Whiteside, T.L. (1996). Alteration in expression and function of signal transducing proteins in tumor-associated T and NK cells in patients with ovarian carcinoma. *Clin. Cancer Res*. **2**, 161–173.

Lai, S.Y., Molden, J. and Goldsmith, M.A. (1997). Shared γ(c) subunit within the human interleukin-7 receptor complex. A molecular basis for the pathogenesis of X-linked severe combined immunodeficiency. *J. Clin. Invest*. **99**, 169–177.

Leclercq, G., De Smedt, M. and Plum, J. (1992). Presence of CD8 α-CD8 β-positive TcR γ/δ thymocytes in the fetal murine thymus and their *in vitro* expansion with interleukin-7. *Eur. J. Immunol*. **22**, 2189–2193.

Lee, G., Namen, A.E., Gillis, S., Ellingsworth, L.R. and Kincade, P.W. (1989). Normal B cell precursors responsive to recombinant murine IL-7 and inhibition of IL-7 activity by transforming growth factor-β. *J. Immunol*. **142**, 3875–3883.

Lee, S.H., Fujita, N., Mashima, T. and Tsuruo, T. (1996). Interleukin-7 inhibits apoptosis of mouse malignant T-lymphoma cells by both suppressing the CPP32-like protease activation and inducing the Bcl-2 expression. *Oncogene* **13**, 2131–2139.

Leonard, W.J., Noguchi, M., Russell, S.M. and McBride, O.W. (1994). The molecular basis of X-linked severe combined immunodeficiency: the role of the interleukin-2 receptor γ chain as a common γ chain, γ c. *Immunol. Rev*. **138**, 61–86.

Levey, D.L. and Srivastava, P.K. (1995). T cells from late tumor-bearing mice express normal levels of p56lck, p59fyn, ZAP-70, and CD3 zeta despite suppressed cytolytic activity. *J. Exp. Med*. **182**, 1029–1036.

Lewis, S.M. (1994). The mechanism of V(D)J joining: lessons from molecular, immunological, and comparative analyses. *Adv. Immunol*. **56**, 27–150.

Li, Y.S., Hayakawa, K. and Hardy, R.R. (1993). The regulated expression of B lineage associated genes during B cell differentiation in bone marrow and fetal liver. *J. Exp. Med*. **178**, 951–960.

Li, Y.S., Wasserman, R., Hayakawa, K. and Hardy, R.R. (1996). Identification of the earliest B lineage stage in mouse bone marrow. *Immunity* **5**, 527–535.

Lin, J.X., Migone, T.S., Tsang, M., Friedmann, M., Weatherbee, J.A., Zhou, L., Yamauchi, A., Bloom, E.T., Mietz, J. and John, S. (1995). The role of shared receptor motifs and common Stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. *Immunity* **2**, 331–339.

Loken, M.R., Shah, V.O., Hollander, Z. and Civin, C.I. (1988). Flow cytometric analysis of normal B lymphoid development. *Pathol. Immunopathol. Res*. **7**, 357–370.

Long, B.W., Witte, P.L., Abraham, G.N., Gregory, S.A. and Plate, J.M. (1995). Apoptosis and interleukin 7 gene expression in chronic B-lymphocytic leukemia cells. *Proc. Natl Acad. Sci. U.S.A*. **92**, 1416–1420.

Lu, L., Zhou, Z., Wu, B., Xiao, M., Shen, R.N., Williams, D.E., Kim, Y.J., Kwon, B.S., Ruscetti, S. and Broxmeyer, H.E. (1992). Influence of recombinant human interleukin (IL)-7 on disease progression in mice infected with Friend virus complex. *Int. J. Cancer* **52**, 261–265.

Lupton, S.D., Gimpel, S., Jerzy, R., Brunton, L.L., Hjerrild, K.A., Cosman, D. and Goodwin, R.G. (1990). Characterization of the human and murine IL-7 genes. *J. Immunol*. **144**, 3592–3601.

Lynch, D.H. and Miller, R.E. (1990). Induction of murine lymphokine-activated killer cells by recombinant IL-7. *J. Immunol*. **145**, 1983–1990.

Lynch, D.H. and Miller, R.E. (1994). Interleukin 7 promotes long-term *in vitro* growth of antitumor cytotoxic T lymphocytes with immunotherapeutic efficacy *in vivo*. *J. Exp. Med*. **179**, 31–42.

Lynch, D.H., Namen, A.E. and Miller, R.E. (1991). *In vivo* evaluation of the effects of interleukins 2, 4 and 7 on enhancing the immunotherapeutic efficacy of anti-tumor cytotoxic T lymphocytes. *Eur. J. Immunol*. **21**, 2977–2985.

Lynch, M., Baker, E., Park, L.S., Sutherland, G.R. and Goodwin, R.G. (1992). The interleukin-7 receptor gene is at 5p13. *Hum. Genet*. **89**, 566–568.

MacNeil, I.A., Suda, T., Moore, K.W., Mosmann, T.R. and Zlotnik, A. (1990). IL-10, a novel growth cofactor for mature and immature T cells. *J. Immunol*. **145**, 4167–4173.

Maeurer, M., Zitvogel, L., Elder, E., Storkus, W.J. and Lotze, M.T. (1995). Human intestinal $V\delta1^+$ T cells obtained from patients with colon cancer respond exclusively to SEB but not to SEA. *Nat. Immun*. **14**, 188–197.

Maeurer, M.J., Martin, D., Walter, W., Liu, K., Zitvogel, L., Halusczcak, K., Rabinowich, H., Duquesnoy, R., Storkus, W. and Lotze, M.T. (1996). Human intestinal $V\delta1^+$ lymphocytes recognize tumor cells of epithelial origin. *J. Exp. Med*. **183**, 1681–1696.

Maeurer, M.J., Walter, W., Martin, D., Zitvogel, L., Elder, E., Storkus, W. and Lotze, M.T. (1997). Interleukin-7 (IL-7) in colorectal cancer: IL-7 is produced by tissues from colorectal cancer and promotes preferential expansion of tumour infiltrating lymphocytes. *Scand. J. Immunol*. **45**, 182–192.

Maki, K., Sunaga, S., Komagata, Y., Kodaira, Y., Mabuchi, A., Karasuyama, H., Yokomuro, K., Miyazaki, J.I. and Ikuta, K. (1996). Interleukin 7 receptor-deficient mice lack $\gamma\delta$ T cells. *Proc. Natl Acad. Sci. U.S.A*. **93**, 7172–7177.

Makrynikola, V., Kabral, A. and Bradstock, K.F. (1991). Effects of recombinant human cytokines on precursor-B acute lymphoblastic leukemia cells. *Exp. Hematol*. **19**, 674–679.

Maraskovsky, E., Teepe, M., Morrissey, P.J., Braddy, S., Miller, R.E., Lynch, D.H. and Peschon, J.J. (1996). Impaired survival and proliferation in IL-7 receptor-deficient peripheral T cells. *J. Immunol*. **157**, 5315–5323.

Maric, D., Kaiserlian, D. and Croitoru, K. (1996). Intestinal epithelial cell line induction of T cell differentiation from bone marrow precursors. *Cell Immunol*. **172**, 172–179.

Matsuda, M., Petersson, M., Lenkei, R., Taupin, J.L., Magnusson, I., Mellstedt, H., Anderson, P. and Kiessling, R. (1995). Alterations in the signal-transducing molecules of T cells and NK cells in colorectal tumor-infiltrating, gut mucosal and peripheral lymphocytes: correlation with the stage of the disease. *Int. J. Cancer* **61**, 765–772.

Matsue, H., Bergstresser, P.R. and Takashima, A. (1993a). Keratinocyte-derived IL-7 serves as a growth factor for dendritic epidermal T cells in mice. *J. Immunol*. **151**, 6012–6019.

Matsue, H., Bergstresser, P.R. and Takashima, A. (1993b). Reciprocal cytokine-mediated cellular interactions in mouse epidermis: promotion of $\gamma\delta$ T-cell growth by IL-7 and TNF α and inhibition of keratinocyte growth by γ IFN. *J. Invest. Dermatol*. **101**, 543–548.

Matsuzaki, Y., Nakayama, K., Tomita, T., Isoda, M., Loh, D.Y. and Nakauchi, H. (1997). Role of bcl-2 in the development of lymphoid cells from the hematopoietic stem cell. *Blood* **89**, 853–862.

Matthews, W., Jordan, C.T., Wiegand, G.W., Pardoll, D. and Lemischka, I.R. (1991). *Cell* **65**, 1143–1152.

McBride, W.H., Thacker, J.D., Comora, S., Economou, J.S., Kelley, D., Hogge, D., Dubinett, S.M. and Dougherty, G.J. (1992). Genetic modification of a murine fibrosarcoma to produce interleukin 7 stimulates host cell infiltration and tumor immunity. *Cancer Res*. **52**, 3931–3937.

McKearn, J.P., McCubrey, J. and Fagg, B. (1985). Enrichment of hematopoietic precursor cells and cloning of multipotential B-lymphocyte precursors. *Proc. Natl Acad. Sci. U.S.A*. **82**, 7414–7418.

McNiece, I.K., Langley, K.E. and Zsebo, K.M. (1991). The role of recombinant stem cell factor in early B cell development. Synergistic interaction with IL-7. *J. Immunol*. **146** 3785–3790.

Mehler, M.F., Rozental, R., Dougherty, M., Spray, D.C. and Kessler, J.A. (1993). Cytokine regulation of neuronal differentiation of hippocampal progenitor cells. *Nature* **362**, 62–65.

Mehrotra, P.M., Grant, A.J. and Siegel, J.P. (1995). Synergistic effects of IL-7 and IL-12 on human T-cell activation. *J. Immunol*. **154**, 5093–5102.

Melhem, M.F., Meisler, A.I., Saito, R., Finley, G.G., Hockman, H.R. and Koski, R.A. (1993). Cytokines in inflammatory malignant fibrous histiocytoma presenting with leukemoid reaction. *Blood* **82**, 2038–2044.

Mertsching, E., Burdet, C. and Ceredig, R. (1995). IL-7 transgenic mice: analysis of the role of IL-7 in the differentiation of thymocytes *in vivo* and *in vitro*. *Int. Immunol*. **7**, 401–414.

Mertsching, E., Grawunder, U., Meyer, V., Rolink, T. and Ceredig, R. (1996). Phenotypic and functional analysis of B lymphopoiesis in interleukin-7-transgenic mice: expansion of pro/pre-B cell number and persistence of B lymphocyte development in lymph nodes and spleen. *Eur. J. Immunol*. **26**, 28–33.

Michaelson, M.D., Mehler, M.F., Xu, H., Gross, R.E. and Kessler, J.A. (1996). Interleukin-7 is trophic for embryonic neurons and is expressed in developing brain. *Dev. Biol*. **179**, 251–263.

Miller, A.R., McBride, W.H., Dubinett, S.M., Dougherty, G.J., Thacker, J.D., Shau, H., Kohn, D.B., Moen, R.C., Walker, M.J. and Chiu, R. (1993). Transduction of human melanoma cell lines with the human interleukin-7 gene using retroviral-mediated gene transfer: comparison of immunologic properties with interleukin-2. *Blood* **82**, 3686–3694.

Miyaji, C., Watanabe, H., Osman, Y., Kuwano, Y. and Abo, T. (1996). A comparison of proliferative response to IL-7 and expression of IL-7 receptors in intermediate TCR cells of the liver, spleen, and thymus. *Cell. Immunol*. **169**, 159–165.

Mizoguchi, H., O'Shea, J.J., Longo, D.L., Loeffler, C.M., McVicar, D.W. and Ochoa, A.C. (1992). Alterations in signal transduction molecules in T lymphocytes from tumor-bearing mice. *Science* **258**, 1795–1798.

Molina, T.J., Kishihara, K., Siderovski, D.P., van Ewijk, W., Narendran, A., Timms, E., Wakeham, A., Paige, C.J., Hartmann, K.U., Veillette, A., Davidson, D. and Mak, T.W. (1992). Profound block in thymocyte development in mice lacking p56lck. *Nature* **357**, 161–164.

Möller, P., Bohm, M., Czarnetszki, B.M. and Schadendorf, D. (1996). Interleukin-7. Biology and implications for dermatology. *Exp. Dermatol*. **5**, 129–137.

Montgomery, R.A. and Dallman, M.J. (1991). Analysis of cytokine gene expression during fetal thymic ontogeny using the polymerase chain reaction. *J. Immunol*. **147**, 554–560.

Moore, T.A., von Freeden-Jeffrey, U., Murray, R. and Zlotnik, A. (1995). *Ninth International Congress on Immunology*, Abstract 441, p. 75.

Moore, T.A., von Freeden-Jeffrey, U., Murray, R. and Zlotnik, A. (1996). Inhibition of $\gamma\delta$ T cell development and early thymocyte maturation in IL-7$^{-/-}$ mice. *J. Immunol*. **157**, 2366–2373.

Moran, P.A., Diegel, M.L., Sias, J.C., Ledbetter, J.A. and Zarling, J.M. (1993). Regulation of HIV production by blood mononuclear cells from HIV-infected donors: I. Lack of correlation between HIV-1 production and T cell activation. *AIDS Res. Hum. Retroviruses* **9**, 455–464.

Morrow, M.A., Lee, G., Gillis, S., Yancopoulos, G.D. and Alt, F.W. (1992). Interleukin-7 induces N-myc and c-myc expression in normal precursor B lymphocytes. *Genes Dev*. **6**, 61–70.

Muegge, K., Vila, M.P. and Durum, S.K. (1994). Interleukin-7: a cofactor for V(D)J rearrangement of the T cell receptor β gene. *Science* **261**, 93–95.

Murphy, W.J., Back, T.C., Conlon, K.C., Komschlies, K.L., Ortaldo, J.R., Sayers, T.J., Wiltrout, R.H. and Longo, D.L. (1993). Antitumor effects of interleukin-7 and adoptive immunotherapy on human colon carcinoma xenografts. *J. Clin. Invest*. **92**, 1918–1924.

Musso, T., Johnston, J.A., Linnekin, D., Varesio, L., Rowe, T.K., O'Shea, J.J. and McVicar, D.W. (1995). Regulation of JAK3 expression in human monocytes: phosphorylation in response to interleukins 2, 4, and 7. *J. Exp. Med*. **181**, 1425–1431.

Nakagomi, H., Petersson, M., Magnusson, I., Juhlin, C., Matsuda, M., Mellstedt, H., Taupin, J.L., Vivier, E., Anderson, P. and Kiessling, R. (1993). Decreased expression of the signal-transducing zeta chains in tumor-infiltrating T-cells and NK cells of patients with colorectal carcinoma. *Cancer Res*. **53**, 5610–5612.

Namen, A.E., Lupton, S., Hjerrild, K., Wignall, J., Mochizuki, D.Y., Schmierer, A., Mosley, B., March, C.J., Urdal, D. and Gillis, S. (1988a). Stimulation of B-cell progenitors by cloned murine interleukin-7. *Nature* **333**, 571–573.

Namen, A.E., Schmierer, A.E., March, C.J., Overell, R.W., Park, L.S., Urdal, D.L. and Mochizuki, D.Y. (1988b). B cell precursor growth-promoting activity. Purification and characterization of a growth factor active on lymphocyte precursors. *J. Exp. Med*. **167**, 988–1002.

Namikawa, R., Muench, M.O., de Vries, J.E. and Roncarolo, M.G. (1996). The FLK2/FLT3 ligand synergizes with interleukin-7 in promoting stromal-cell-independent expansion and differentiation of human fetal pro-B cells *in vitro*. *Blood* **87**, 1881–1890.

Naume, B. and Espevik, T. (1991). Effects of IL-7 and IL-2 on highly enriched CD56$^+$ natural killer cells. A comparative study. *J. Immunol*. **147**, 2208–2214.

Naume, B., Gately, M. and Espevik, T. (1992). A comparative study of IL-12 (cytotoxic lymphocyte maturation factor)-, IL-2-, and IL-7-induced effects on immunomagnetically purified CD56$^+$ NK cells. *J. Immunol*. **148**, 2429–2436.

Naume, B., Johnsen, A.C., Espevik, T. and Sundan, A. (1993). Gene expression and secretion of cytokines and

cytokine receptors from highly purified CD56$^+$ natural killer cells stimulated with interleukin-2, interleukin-7 and interleukin-12. *Eur. J. Immunol.* **23**, 1831–1838.

Noguchi, M., Nakamura, Y., Russell, S.M., Ziegler, S.F., Tsang, M., Cao, X. and Leonard, W.J. (1993). Interleukin-2 receptor γ chain: a functional component of the interleukin-7 receptor. *Science* **262**, 1877–1880.

Nosaka, T., van Deursen, J.M., Tripp, R.A., Thierfelder, W.E., Witthuhn, B.A., McMickle, A.P., Doherty, P.C., Grosveld, G.C. and Ihle, J.N. (1995). Defective lymphoid development in mice lacking Jak3. *Science* **270**, 800–802.

Oettinger, M.A., Schatz, D.G., Gorka, C. and Baltimore, D. (1990). *RAG-1* and *RAG-2*, adjacent genes that synergistically activate V(D)J recombination. *Science* **248**, 1517–1523.

Okazaki, H., Ito, M., Sudo, T., Hattori, M., Kano, S., Katsura, Y. and Minato, N. (1989). IL-7 promotes thymocyte proliferation and maintains immunocompetent thymocytes bearing α/β or $\gamma\delta$ T-cell receptors *in vitro*: synergism with IL-2. *J. Immunol.* **143**, 2917–2922.

Oltz, E.M., Yancopoulos, G.D., Morrow, M.A., Rolink, A., Lee, G., Wong, F., Kaplan, K., Gillis, S., Melchers, F. and Alt, F.W. (1992). A novel regulatory myosin light chain gene distinguishes pre-B cell subsets and is IL-7 inducible. *EMBO J.* **11**, 2759–2767.

Ono, M., Ariizumi, K., Bergstresser, P.R. and Takashima, A. (1996). IL-7 upregulates T cell receptor/CD3 expression by cultured dendritic epidermal T cells. *J. Dermatol. Sci.* **11**, 89–96.

Oosterwegel, M.A., Haks, M.C., von Freeden-Jeffrey, U., Murray, R. and Kruisbeek, A.M. (1997). Induction of TCR gene rearrangements in uncommitted stem cells by a subset of IL-7 producing, MHC class-II-expressing thymic stromal cells. *Immunity* **6**, 351–360.

Orlic, D., Fischer, R., Nishikawa, S., Nienhuis, A.W. and Bodine, D.M. (1993). Purification and characterization of heterogeneous pluripotent hematopoietic stem cell populations expressing high levels of c-kit receptor. *Blood* **82**, 762–770.

Page, T.H., Willcocks, J.L., Taylor Fishwick, D.A. and Foxwell, B.M. (1993). Characterization of a novel high affinity human IL-7 receptor. Expression on T cells and association with IL-7 driven proliferation. *J. Immunol.* **151**, 4753–4763.

Page, T.H., Lali, F.V., Groome, N. and Foxwell, B.M.J. (1997). Association of the common γ-chain with the human IL-7 receptor is modulated by T cell activation. *J. Immunol.* **158**, 5727–5735.

Pandrau Garcia, D., de Saint Vis, B., Saeland, S., Renard, N., Ho, S., Moreau, I., Banchereau, J. and Galizzi, J.P. (1994). Growth inhibitory and agonistic signals of interleukin-7 (IL-7) can be mediated through the CDw127 IL-7 receptor. *Blood* **83**, 3613–3619.

Park, L.S., Friend, D.J., Schmierer, A.E., Dower, S.K. and Namen, A.E. (1990). Murine interleukin 7 (IL-7) receptor. Characterization on an IL-7-dependent cell line. *J. Exp. Med.* **171**, 1073–1089.

Park, S.Y., Saijo, K., Takahashi, T., Osawa, M., Arase, H., Hirayama, N., Miyake, K., Nakauchi, H., Shirasawa, T. and Saito, T. (1995). Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. *Immunity* **3**, 771–782.

Perumal, N.B., Kenniston, T.W., Jr., Tweardy, D.J., Dyer, K.F., Hoffman, R., Peschon, J. and Appasamy, P.M. (1997). *J. Immunol.* **158**, 5744–5750.

Peschon, J.J., Morrissey, P.J., Grabstein, K.H., Ramsdell, F.J., Maraskovsky, E., Gliniak, B.C., Park, L.S., Ziegler, S.F., Williams, D.E. and Ware, C.B. (1994). Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. *J. Exp. Med.* **180**, 1955–1960.

Peschon, J.J., Gliniak, B.C., Morissey, P. and Maraskowsky, E. (1997). In *Cytokine Knockouts* (eds S.K. Durak and U. Muegge), Humana Press, Totowo, New Jersey.

Pleiman, C.M., Gimpel, S.D., Park, L.S., Harada, H., Taniguchi, T. and Ziegler, S.F. (1991). *Mol. Cell. Biol.* **11**, 3052–3059.

Plum, J., De Smedt, M. and Leclercq, G. (1993). Exogenous IL-7 promotes the growth of CD3$^-$ CD4$^-$ CD8$^-$ CD44$^+$ CD25$^{+/-}$ precursor cells and blocks the differentiation pathway of TCR-$\alpha\beta$ cells in fetal thymus organ culture. *J. Immunol.* **150**, 2706–2716.

Poussier, P. and Julius, M. (1994). Thymus independent T cell development and selection in the intestinal epithelium. *Annu. Rev. Immunol.* **12**, 521–553.

Prieyl, J.A. and LeBien, T.W. (1996). Interleukin 7 independent development of human B cells. *Proc. Natl Acad. Sci. U.S.A.* **93**, 10348–10353.

R&D Systems (1994). Levels of IL-1, IL-3, IL-6, IL-7 TNF, and basic FGF in normal blood and urine. In *Cytokine Bulletin*, R&D Systems, Minneapolis, p. 3.

Rabinowich, H., Banks, M., Reichert, T., Logan, T.F., Kirkwood, J.M. and Whiteside, T.L. (1996). Expression

and activity of signalling molecules in T lymphocytes obtained from patients with metastatic melanoma before and after interleukin-2 therapy. *Clin. Cancer Res*. **2**, 1263–1274.

Ray, R.J., Paige, C.J., Furlonger, C., Lyman, S.D. and Rottapel, R. (1996). Flt3 ligand supports the differentiation of early B cell progenitors in the presence of interleukin-11 and interleukin-7. *Eur. J. Immunol*. **26**, 1504–1510.

Reinecker, H.C. and Podolsky, D.K. (1995). Human intestinal epithelial cells express functional cytokine receptors sharing the common γc chain of the interleukin 2 receptor. *Proc. Natl Acad. Sci. U.S.A*. **92**, 8353–8357.

Rich, B.E., Campos Torres, J., Tepper, R.I., Moreadith, R.W. and Leder, P. (1993). Cutaneous lymphoproliferation and lymphomas in interleukin 7 transgenic mice. *J. Exp. Med*. **177**, 305–316.

Rocha, B., Vassalli, P. and Guy Grand, D. (1994). Thymic and extrathymic origins of gut intraepithelial lymphocyte populations in mice. *J. Exp. Med*. **180**, 681–686.

Rosnet, O., Marchetto, S., deLapeyriere, O. and Birnbaum, D. (1991). Murine Flt3, a gene encoding a novel tyrosine kinase receptor of the PDGFR/CSF1R family. *Oncogene* **6**, 1641–1650.

Russell, S.M., Keegan, A.D., Harada, N., Nakamura, Y., Noguchi, M., Leland, P., Friedmann, M.C., Miyajima, A., Puri, R.K. and Paul, W.E. (1993). Interleukin-2 receptor γ chain: a functional component of the interleukin-4 receptor. *Science* **262**, 1880–1883.

Russell, S.M., Johnston, J.A., Noguchi, M., Kawamura, M., Bacon, C.M., Friedmann, M., Berg, M., McVicar, D.W., Witthuhn, B.A. and Silvennoinen, O. (1994). Interaction of IL-2R β and γc chains with Jak1 and Jak3: implications for XSCID and XCID. *Science* **266**, 1042–1045.

Ryan, D.H., Nuccie, B.L., Ritterman, I., Liesveld, J.L. and Abboud, C.N. (1994). Cytokine regulation of early human lymphopoiesis. *J. Immunol*. **152**, 5250–5258.

Sakata, T., Iwagami, S., Tsuruta, Y., Teraoka, H., Tatsumi, Y., Kita, Y., Nishikawa, S., Takai, Y. and Fujiwara, H. (1990). Constitutive expression of interleukin-7 mRNA and production of IL-7 by a cloned murine thymic stromal cell line. *J. Leukoc. Biol*. **48**, 205–212.

Salvadori, S., Gansbacher, B., Pizzimenti, A.M. and Zier, K.S. (1994). Abnormal signal transduction by T cells of mice with parental tumors is not seen in mice bearing IL-2-secreting tumors. *J. Immunol*. **153**, 5176–5182.

Sartono, E., Kruize, Y.C., Partono, F., Kurniawan, A., Maizels, R.M. and Yazdanbakhsh, M. (1995). Specific T cell unresponsiveness in human filariasis: diversity in underlying mechanisms. *Parasite Immunol*. **17**, 587–594.

Schlossman, S.F., Boumsell, L., Gilks, W., Harlan, J.M., Kishimoto, T., Morimoto, C., Ritz, J., Shaw, S., Silverstein, R.L., Springer, T.A., Tedder, T.F. and Todd, R.F. (1994). CD antigens 1993. *J. Immunol*. **152**, 1.

Schorle, H., Holtschke, T., Hunig, T., Schimpl, A. and Horak, I. (1991). Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. *Nature* **352**, 621–624.

Seckinger, P. and Fougereau, M. (1994). Activation of src kinases in human pre-B cells by IL-7. *J. Immunol*. **153**, 97–109.

Shand, R.F. and Betlach, M.C. (1991). Expression of the bop gene cluster of *Halobacterium halobium* is induced by low oxygen tension and by light. *J. Bacteriol*. **173**, 4692–4699.

Sharfe, N., Dadi, H.K. and Roifman, C.M. (1995). JAK3 protein tyrosine kinase mediates interleukin-7-induced activation of phosphatidylinositol-3′ kinase. *Blood* **86**, 2077–2085.

Sharma, S., Wang, J., Huang, M., Paul, R.W., Lee, P., McBride, W.H., Economou, J.S., Roth, M.D., Kiertscher, S.M. and Dubinett, S.M. (1996). Interleukin-7 gene transfer in non-small-cell lung cancer decreases tumor proliferation, modifies cell surface molecule expression, and enhances antitumor reactivity. *Cancer Gene Ther*. **3**, 302–313.

Sherwood, P.J. and Weissman, I.L. (1990). The growth factor IL-7 induces expression of a transformation-associated antigen in normal pre-B cells. *Int. Immunol*. **2**, 399–406.

Sica, D., Rayman, P., Stanley, J., Edinger, M., Tubbs, R.R., Klein, E., Bukowski, R. and Finke, J.H. (1993). Interleukin 7 enhances the proliferation and effector function of tumor-infiltrating lymphocytes from renal-cell carcinoma. *Int. J. Cancer* **53**, 941–947.

Sieling, P.A., Sakimura, L., Uyemura, K., Yamamura, M., Oliveros, J., Nickoloff, B.J., Rea, T.H. and Modlin, R.L. (1995). IL-7 in the cell-mediated immune response to a human pathogen. *J. Immunol*. **154**, 2775–2783.

Sillevis Smitt, J.H., Weening, R.S., Krieg, S.R. and Bos, J.D. (1992). The skin in severe combined immunodeficiency: a case with transient cutaneous presence of γ/δ ($TRC1^+$) T cells. *Br. J. Dermatol*. **127**, 281–285.

Skeen, M.J. and Ziegler, H.K. (1993). Induction of murine peritoneal γ/δ T cells and their role in resistance to bacterial infection. *J. Exp. Med*. **178**, 971–984.

Skjonsberg, C., Erikstein, B.K., Smeland, E.B., Lie, S.O., Funderud, S., Beiske, K. and Blomhoff, H.K. (1991). Interleukin-7 differentiates a subgroup of acute lymphoblastic leukemias. *Blood* **77**, 2445–2450.

Slieker, W.A., van der Loo, J.C., de Rijk de Bruijn, M.F., Godfrey, D.I., Leenen, P.J. and van Ewijk, W. (1993). ER-MP12 antigen, a new cell surface marker on mouse bone marrow cells with thymus-repopulating ability: II. Thymus-homing ability and phenotypic characterization of ER-MP12-positive bone marrow cells. *Int. Immunol.* **5**, 1099–1107.

Smithgall, M.D., Wong, J.G., Critchett, K.E. and Haffar, O.K. (1996). IL-7 up-regulates HIV-1 replication in naturally infected peripheral blood mononuclear cells. *J. Immunol.* **156**, 2324–2330.

Stall, A.M. and Wells, S.M. (1996). Introduction: B-1 cells: origins and functions. *Semin. Immunol.* **8**, 1–2.

Standiford, T.J., Strieter, R.M., Allen, R.M., Burdick, M.D. and Kunkel, S.L. (1992). IL-7 up-regulates the expression of IL-8 from resting and stimulated human blood monocytes. *J. Immunol.* **149**, 2035–2039.

Stein, P.L., Lee, H.M., Rich, S. and Soriano, P. (1992). pp59fyn mutant mice display differential signaling in thymocytes and peripheral T cells. *Cell* **70**, 741–750.

Steiner, G., Koning, F., Elbe, A., Tschachler, E., Yokoyama, W.M., Shevach, E.M., Stingl, G. and Coligan, J.E. (1988). Characterization of T cell receptors on resident murine dendritic epidermal T cells. *Eur. J. Immunol.* **18**, 1323–1328.

Stephan, R.P., Sanders, V.M. and Witte, P.L. (1996). Stage-specific alterations in murine B lymphopoiesis with age. *Int. Immunol.* **8**, 509–518.

Stephan, R.P., Lill Elghanian, D.A. and Witte, P.L. (1997). Development of B cells in aged mice: decline in the ability of pro-B cells to respond to IL-7 but not to other growth factors. *J. Immunol.* **158**, 1598–1609.

Stötter, H. and Lotze, M.T. (1991). Human lymphokine-activated killer cell activity. Role of IL-2, IL-4, and IL-7. *Arch. Surg.* **126**, 1525–1530.

Stötter, H., Custer, M.C., Bolton, E.S., Guedez, L. and Lotze, M.T. (1991). IL-7 induces human lymphokine-activated killer cell activity and is regulated by IL-4. *J. Immunol.* **146**, 150–155.

Strehlau, J., Pavlakis, M., Lipman, M., Shapiro, M., Vasconcellos, L., Harmon, W. and Strom, T.B. (1997). Quantitative detection of immune activation transcripts as a diagnostic tool in kidney transplantation. *Proc. Natl Acad. Sci. U.S.A.* **94**, 695–700.

Suda, T. and Zlotnik, A. (1991). IL-7 maintains the T cell precursor potential of $CD3^-$ $CD4^-$ $CD8^-$ thymocytes. *J. Immunol.* **146**, 3068–3073.

Suda, T., Okada, S., Suda, J., Miura, Y., Ito, M., Sudo, T., Hayashi, S., Nishikawa, S. and Nakauchi, H. (1989). A stimulatory effect of recombinant murine interleukin-7 (IL-7) on B-cell colony formation and an inhibitory effect of IL-1α. *Blood* **74**, 1936–1941.

Sudo, T., Ito, M., Ogawa, Y., Iizuka, M., Kodama, H., Kunisada, T., Hayashi, S., Ogawa, M., Sakai, K. and Nishikawa, S. (1989). Interleukin 7 production and function in stromal cell-dependent B cell development. *J. Exp. Med.* **170**, 333–338.

Sudo, T., Nishikawa, S., Ohno, N., Akiyama, N., Tamakoshi, M. and Yoshida, H. (1993). Expression and function of the interleukin 7 receptor in murine lymphocytes. *Proc. Natl Acad. Sci. U.S.A.* **90**, 9125–9129.

Sugamura, K., Asao, H., Kondo, M., Tanaka, N., Ishii, N., Ohbo, K., Nakamura, M. and Takeshita, T. (1996). The interleukin-2 receptor γ chain: its role in the multiple cytokine receptor complexes and T cell development in XSCID. *Annu. Rev. Immunol.* **14**, 179–205.

Sutherland, G.R., Baker, E., Fernandez, K.E., Callen, D.F., Goodwin, R.G., Lupton, S., Namen, A.E., Shannon, M.F. and Vadas, M.A. (1989). The gene for human interleukin 7 (IL7) is at 8q12–13. *Hum. Genet.* **82**, 371–372.

Sweeney, E., van der Spek, J. and Murphy, J. (1995). IL-7 receptor-specific cell killing by DAB389-IL-7: a novel agent for the elimination of IL-7 receptor-positive malignant cells. *Fourth International Symposium on Immunotoxins*, p. 74 (abstract).

Szilvassy, S.J. and Cory, S. (1993). Phenotypic and functional characterization of competitive long-term repopulating hematopoietic stem cells enriched from 5-fluorouracil-treated murine marrow. *Blood* **81**, 2310–2320.

Szilvassy, S.J., Lansdorp, P.M., Humphries, R.K., Eaves, A.C. and Eaves, C.J. (1989). Isolation in a single step of a highly enriched murine hematopoietic stem cell population with competitive long-term repopulating ability. *Blood* **74**, 930–939.

Takashima, A. and Bergstresser, P.R. (1996). Cytokine-mediated communication by keratinocytes and Langerhans cells with dendritic epidermal T cells. *Semin. Immunol.* **8**, 333–339.

Takashima, A., Matsue, H., Bergstresser, P.R. and Ariizumi, K. (1995). Interleukin-7-dependent interaction of dendritic epidermal T cells with keratinocytes. *J. Invest. Dermatol.* **105**, 50S–53S.

Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Munakata, H., Nakamura, M. and Sugamura, K. (1992). Cloning of the γ chain of the human IL-2 receptor. *Science* **257**, 379–382.

Talmadge, J.E., Jackson, J.D., Kelsey, L., Borgeson, C.D., Faltynek, C. and Perry, G.A. (1993). T-cell reconstitution by molecular, phenotypic, and functional analysis in the thymus, bone marrow, spleen, and blood following split-dose polychemotherapy and therapeutic activity for metastatic breast cancer in mice. *J. Immunother.* **14**, 258–268.

Tang, J., Nuccie, B.L., Ritterman, I., Liesveld, J.L., Abboud, C.N. and Ryan, D.H. (1997). TGF-β down-regulates stromal IL-7 secretion and inhibits proliferation of human B cell precursors. *J. Immunol.* **159**, 117–125.

Taniguchi, T. and Minami, Y. (1993). The IL-2/IL-2 receptor system: a current overview. *Cell* **73**, 5–8.

Tantawichien, T., Young, L.S. and Bermudez, L.E. (1996). Interleukin-7 induces anti-*Mycobacterium avium* activity in human monocyte-derived macrophages. *J. Infect. Dis.* **174**, 574–582.

Tartour, E., Latour, S., Mathiot, C., Thiounn, N., Mosseri, V., Joyeux, I., D'Enghien, C.D., Lee, R., Debre, B. and Fridman, W.H. (1995). Variable expression of CD3-zeta chain in tumor-infiltrating lymphocytes (TIL) derived from renal-cell carcinoma: relationship with TIL phenotype and function. *Int. J. Cancer* **63**, 205–212.

Tepper, R.I. and Mule, J.J. (1994). Experimental and clinical studies of cytokine gene-modified tumor cells. *Hum. Gene Ther.* **5**, 153–164.

Touw, I., Pouwels, K., van Agthoven, T., van Gurp, R., Budel, L., Hoogerbrugge, H., Delwel, R., Goodwin, R., Namen, A. and Lowenberg, B. (1990). Interleukin-7 is a growth factor of precursor B and T acute lymphoblastic leukemia. *Blood* **75**, 2097–2101.

Trumper, L., Jung, W., Dahl, G., Diehl, V., Gause, A. and Pfreundschuh, M. (1994). Interleukin-7, interleukin-8, soluble TNF receptor, and p53 protein levels are elevated in the serum of patients with Hodgkin's disease. *Ann. Oncol.* **5 (supplement 1)**, 93–96.

Tushinski, R.J., McAlister, I.B., Williams, D.E. and Namen, A.E. (1991). The effects of interleukin 7 (IL-7) on human bone marrow *in vitro*. *Exp. Hematol.* **19**, 749–754.

Uckun, F.M., Tuel Ahlgren, L., Obuz, V., Smith, R., Dibirdik, I., Hanson, M., Langlie, M.C. and Ledbetter, J.A. (1991). Interleukin 7 receptor engagement stimulates tyrosine phosphorylation, inositol phospholipid turnover, proliferation, and selective differentiation to the CD4 lineage by human fetal thymocytes. *Proc. Natl Acad. Sci. U.S.A.* **88**, 6323–6327.

Uehira, M., Matsuda, H., Hikita, I., Sakata, T., Fujiwara, H. and Nishimoto, H. (1993). The development of dermatitis infiltrated by $\gamma\delta$ T cells in IL-7 transgenic mice. *Int. Immunol.* **5**, 1619–1627.

van der Plas, D.C., Smiers, F., Pouwels, K., Hoefsloot, L.H., Lowenberg, B. and Touw, I.P. (1996). Interleukin-7 signaling in human B cell precursor acute lymphoblastic leukemia cells and murine BAF3 cells involves activation of STAT1 and STAT5 mediated via the interleukin-7 receptor α chain. *Leukemia* **10**, 1317–1325.

Van Kerckhove, C., Russell, G.J., Deusch, K., Reich, K., Bhan, A.K., DerSimonian, H. and Brenner, M.B. (1992). Oligoclonality of human intestinal intraepithelial T cells. *J. Exp. Med.* **175**, 57–63.

Veiby, O.P., Lyman, S.D. and Jacobsen, S.E. (1996a). Combined signaling through interleukin-7 receptors and flt3 but not c-kit potently and selectively promotes B-cell commitment and differentiation from uncommitted murine bone marrow progenitor cells. *Blood* **88**, 1256–1265.

Veiby, O.P., Jacobsen, F.W., Cui, L., Lyman, S.D. and Jacobsen, S.E. (1996b). The flt3 ligand promotes the survival of primitive hemopoietic progenitor cells with myeloid as well as B lymphoid potential. Suppression of apoptosis and counteraction by TNF-α and TGF-β. *J. Immunol.* **157**, 2953–2960.

Vellenga, E., Esselink, M.T., Straaten, J., Stulp, B.K., De Wolf, J.T., Brons, R., Giannotti, J., Smit, J.W. and Halie, M.R. (1992). The supportive effects of IL-7 on eosinophil progenitors from human bone marrow cells can be blocked by anti-IL-5. *J. Immunol.* **149**, 2992–2995.

Veis, D.J., Sorenson, C.M., Shutter, J.R. and Korsmeyer, S.J. (1993). Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell* **75**, 229–240.

Vella, A., Teague, T.K., Ihle, J., Kappler, J. and Marrack, P. (1997). Interleukin 4 (IL-4) or IL-7 prevents the death of resting T-cells: Stat6 is probably not required for the effect of IL-4. *J. Exp. Med.* **186**, 325–330.

Vidal, K., Grosjean, I., Evillard, J.P., Gespach, C. and Kaiserlian, D. (1993). Immortalization of mouse intestinal epithelial cells by the SV40-large T gene. Phenotypic and immune characterization of the MODE-K cell line. *J. Immunol. Methods* **166**, 63–73.

von Freeden-Jeffrey, U., Vieira, P., Lucian, L.A., McNeil, T., Burdach, S.E. and Murray, R. (1995). Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. *J. Exp. Med.* **181**, 1519–1526.

Watanabe, M., Ueno, Y., Yajima, T., Iwao, Y., Tsuchiya, M., Ishikawa, H., Aiso, S., Hibi, T. and Ishii, H. (1995). Interleukin 7 is produced by human intestinal epithelial cells and regulates the proliferation of intestinal mucosal lymphocytes. *J. Clin. Invest.* **95**, 2945–2953.

Watanabe, Y., Mazda, O., Aiba, Y., Iwai, K., Gyotoku, J., Ideyama, S., Miyazaki, J. and Katsura, Y. (1992). A

murine thymic stromal cell line which may support the differentiation of $CD4^{-}8^{-}$ thymocytes into $CD4^{+}8^{-}$ $\alpha\beta$ T cell receptor positive T cells. *Cell. Immunol.* **142**, 385–397.

Watson, J.D., Morrissey, P.J., Namen, A.E., Conlon, P.J. and Widmer, M.B. (1989). Effect of IL-7 on the growth of fetal thymocytes in culture. *J. Immunol.* **143**, 1215–1222.

Welch, P.A., Namen, A.E., Goodwin, R.G., Armitage, R. and Cooper, M.D. (1989). Human IL-7: a novel T cell growth factor. *J. Immunol.* **143**, 3562–3567.

Widmer, M.B., Morrissey, P.J., Namen, A.E., Voice, R.F. and Watson, J.D. (1990). Interleukin 7 stimulates growth of fetal thymic precursors of cytolytic cells: induction of effector function by interleukin 2 and inhibition by interleukin 4. *Int. Immunol.* **2**, 1055–1061.

Wiles, M.V., Ruiz, P. and Imhof, B.A. (1992). Interleukin-7 expression during mouse thymus development. *Eur. J. Immunol.* **22**, 1037–1042.

Williams, D.E., Eisenman, J., Baird, A., Rauch, C., Van Ness, K., March, C.J., Park, L.S., Martin, U., Mochizuki, D.Y., Boswell, H.S., Burgess, G.S., Cosman, D. and Lyman, S.D. (1990). Identification of a ligand for the c-*kit* proto-oncogene. *Cell* **63**, 167–174.

Wineman, J.P., Gilmore, G.L., Gritzmacher, C., Torbett, B.E. and Muller Sieburg, C.E. (1992). CD4 is expressed on murine pluripotent hematopoietic stem cells. *Blood* **80**, 1717–1724.

Witte, P.L., Frantsve, L.M., Hergott, M. and Rahbe, S.M. (1993). Cytokine production and heterogeneity of primary stromal cells that support B lymphopoiesis. *Eur. J. Immunol.* **23**, 1809–1817.

Wolf, M.L., Weng, W.K., Stieglbauer, K.T., Shah, N. and LeBien, T.W. (1993). Functional effect of IL-7-enhanced CD19 expression on human B cell precursors. *J. Immunol.* **151**, 138–148.

Xu, S., Ariizumi, K., Edelbaum, D., Bergstresser, P.R. and Takashima, A. (1995). Cytokine-dependent regulation of growth and maturation in murine epidermal dendritic cell lines. *Eur. J. Immunol.* **25**, 1018–1024.

Yamada, N., Wakugawa, M., Kuwata, S., Nakagawa, H. and Tamaki, K. (1996). Changes in eosinophil and leukocyte infiltration and expression of IL-6 and IL-7 messenger RNA in mite allergen patch test reactions in atopic dermatitis. *J. Allergy Clin. Immunol.* **98**, S201–S206.

Yamaoka, Y., Kita, M., Kodama, T., Sawai, N., Kashima, K. and Imanishi, J. (1995). Expression of cytokine mRNA in gastric mucosa with *Helicobacter pylori* infection. *Scand. J. Gastroenterol.* **30**, 1153–1159.

Yasunaga, M., Wang, F., Kunisada, T. and Nishikawa, S. (1995). Cell cycle control of c-kit^{+}IL-$7R^{+}$ B precursor cells by two distinct signals derived from IL-7 receptor and c-kit in a fully defined medium. *J. Exp. Med.* **182**, 315–323.

Yoshioka, R., Shimizu, S., Tachibana, J., Hirose, Y., Fukutoku, M., Takeuchi, Y., Sugai, S., Takiguchi, T. and Konda, S. (1992). Interleukin-7 (IL-7)-induced proliferation of $CD8^{+}$ T-chronic lymphocytic leukemia cells. *J. Clin. Immunol.* **12**, 101–106.

Young, J.C., Gishizky, M.L. and Witte, O.N. (1991). Hyperexpression of interleukin-7 is not necessary or sufficient for transformation of a pre-B lymphoid cell line. *Mol. Cell Biol.* **11**, 854–863.

Yron, I., Wood, T.A. Jr., Spiess, P.J. and Rosenberg, S.A. (1980). *In vitro* growth of murine T cells. V. The isolation and growth of lymphoid cells infiltrating syngeneic solid tumors. *J. Immunol.* **125**, 238–245.

Zea, A.H., Cutrie, B.D., Longo, D.L., Alvord, W.G., Strobl, S.L., Mizoguchi, H., Creekmore, S.P., O'Shea, J.J., Powers, G.C., Urba, W.J. and Ochoa, A.C. (1995). Alterations in T cell receptor and signal transduction molecules in melanoma patients. *Clin. Cancer Res.* **1**, 1327–1335.

Zeng, Y.X., Takahashi, H., Shibata, M. and Hirokawa, K. (1994). JAK3 Janus kinase is involved in interleukin 7 signal pathway. *FEBS Lett.* **353**, 289–293.

Ziegler, S.F., Tough, T.W., Franklin, T.L., Armitage, R.J. and Alderson, M.R. (1991). Induction of macrophage inflammatory protein-1β gene expression in human monocytes by lipopolysaccharide and IL-7. *J. Immunol.* **147**, 2234–2239.

Zharhary, D. (1994). In *Handbook of B and T lymphocytes* (ed. E.C. Snow), Academic Press, San Diego, CA, p. 91.